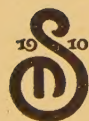


LARS MUNCK

Improvement of nutritional value in cereals



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INAUGURAL DISSERTATION

By due permission of the Faculty of Science
of the University of Lund
to be publicly defended in the lecture hall
of the Institute of Genetics
November 24, 1972, at 9 a.m.,
for the degree of Doctor of Philosophy

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(Received September 6, 1972)

This investigation is principally aimed at exemplifying the developmental sources (italicized below) that are the requisites to obtain an adequate nutritional role for cereals in feeds and foods.

Barley for feed purposes is given central attention and is compared with wheat, rye, ryewheat and oats. The importance of *basic research* in plant and animal sciences is briefly reviewed, and also complemented with experimental results. Development of reliable, rapid and inexpensive *screening methods* is stressed and exemplified by a feeding test with mice and a dye-binding (DBC) method for estimating lysine. Screening analyses for quality characters in cereals are used in *inventories* studying cereals, feed supplements, and complete commercial feeds. The information obtained is tentatively evaluated and is used for promulgating a provisional *development programme* for Swedish conditions. The research that has been carried out for the *realization of the programme* is discussed with attention focused particularly on the development of high-lysine barleys and a high-temperature drying process for cereals for livestock feeds.

A high-protein, high-lysine barley line (Hiproly) was recovered from the World Barley Collection employing the DBC method. A recessive gene (*lys*) was found in Hiproly that increases lysine and other essential amino acids in the endosperm independently of protein content. Genetical, plant-physiological, biochemical, ultrastructural and animal nutritional aspects of high-lysine genes in barley and in maize are extensively discussed along with concomitant analyses of the *lys* gene.

Problems involving grain deterioration and its prevention in Scandinavian cereal production are considered. In addition, the effect of heat on the availability of lysine in cereals during storage, drying and baking is experimentally studied. On the basis of the observations and results, a full-scale, rapid, high-temperature drying method has been developed that is adopted in agriculture in Sweden.

The results are summarized and confronted with the operational concept of nutritional value. The needs for retrieval of information from research and from the applied sphere of cereal utilization — all for optimal exploitation by plant breeders — are discussed for conditions prevalent in Sweden, on the one hand, and in the developing countries, on the other. Research data from basic sciences as well as information from regional inventories on technological, ecological and social conditions have to be co-ordinated in a synthesis in science specific for each country. New solutions must be transferred to the individual and group level of people who can use them as selection criteria favouring survival and quality in economy and life.

The impetus and inspiration for this investigation, which has been in progress since the early 1960's (MUNCK 1962), have been engendered by the urgency to promulgate directive and sustained

goals for plant breeding. The experimental and applied data produced in co-operation with a number of co-workers (p. 120) have been systematically collected and collated in presenting a

methodology of interdisciplinary interpretation, the needs of which were expressed by MUNCK (1964 d):

"It is difficult to make optimal progress in the work of developing the nutritional value of cereals if it be considered as a pure animal-nutritional or agricultural or technological or biochemical or plant breeding subject. There is only one way to obtain optimal results — merely to consider it as a complex problem of its own where all possible methods are allowed to compete in its development."

The material is presented in a survey in Chapter VII (p. 113) referring to experimental results and specialized discussions in Chapters I—VI.

I. Defining the use of the analytical potential

1. Increasing the yield of nutrients

In the different production chains where cereals, e.g., bread wheat, malting barley and feed barley are used, the breeder's primary concern is the grain-producing farmer, through whom the desiderata from the rest of the production chains (e.g., household consumers, bakeries, malters and swine producers) slowly penetrate in a simplified form. The grain farmers select the cereal varieties from the commercial seed market, with yield as the most important criterion, implicating that the commodity in question would give the highest possible monetary return. But even in the few instances where a premium is set for quality (viz., protein for bread and feed), yield is still the dominating economic factor for the farmer. A consideration of a plant breeder's list of priorities in rice breeding reflects this evaluation (BEACHELL and KHUSH 1969):

- (1) Nitrogen responsiveness conditioned by a dwarf gene and favourable plant type
- (2) Photoperiod insensitivity
- (3) Resistance to major diseases and insect pests
- (4) Different growth durations
- (5) Resistance to leaf damage during typhoons
- (6) Slow leaf senescence
- (7) A proper threshability (to avoid shattering)

- (8) Grain dormancy
- (9) Grain size and appearance
- (10) Milling yield
- (11) Eating and cooking quality
- (12) Protein content
- (13) Tolerance to cold weather
- (14) Ability to withstand deep water

In this list, points 1, 2, 3, 6 and 7 directly reflect yield; points 2 and 4 are characters of importance for multicropping; and points 5, 8, 13 and 14 represent characteristics for weather resistance, and thus are also important factors for a reliable yield. Consumers' quality points 9, 10, 11 and 12 are taken care of in the breeding work, but certainly do not have the most urgent priorities. The list also reflects the important measures that are taken to adapt the cereal plant to the environment. The use of nitrogen dressings is thus exploited by the plant breeder through introducing dwarfing genes that enable high yield by inhibiting lodging. Changes in agricultural technology through the invention and introduction of new machines, fertilizers and pesticides keep the breeders busy adjusting their plant materials to the new environments.

During the 19th century, the soil fertility requirements for plant growth were gradually defined (see review by DE WIT 1968) as a result of investigations by VON WULFEN (1823), LAWES (1847) and LIEBIG (1855). The dynamics of plant nutrition are illustrated by DE WIT (1968). One hectare of poor land yields 1000 kg of grass dry matter containing 25 kg of plant minerals, whereas one hectare of good, fertilized land produces 10,000 kg of grass containing 900 kg of minerals.

Photosynthesis is dependent upon insolation and independent of CO_2 concentration at low light intensities, whereas the opposite is the case at high light intensities. BOYSEN-JENSEN (1932) estimated the optimal gross photosynthesis of a crop surface to be 300 kg carbohydrates per hectare daily. The potential growth rate was calculated to be 200 kg per hectare daily. The difference to gross photosynthesis is due to respiration and root growth. These estimations are still valid for a variety of plants, such as algae, grasses, cereals, legumes and root crops, provided that an optimal supply of water and nutrients is available. The daily yields correspond for north-western European conditions to a



theoretical maximal yield of about 50,000 kg dry substance per growing season and hectare, or about 25,000 kg above ground parts, or about 10,000 kg in the form of cereal seeds. The influence of weather and canopy pattern seems to be surprisingly small on the daily yield increase in dry matter in optimized trials (DE WIT 1968).

Even if it is occasionally possible to obtain yields at the limits theoretically possible, a stable, high-yield level is difficult to maintain in practice with large-scale monocultures because of negative plant interactions between pests, fertilizers and climate. The main impact of research in plant physiology (nutrition) on plant breeding has been an indirect one, leading to a drastic change of the plant environment engendering new adaptational problems for the plant breeder. In synthesizing disease- and lodging-resistant varieties, plant breeders have increased yield stability of edible plant parts and thus have greatly exploited the new potentials for gain created by environmental manipulation.

If we compare today in Sweden 50-year-old barley varieties with our present high-yielding ones under modern growing conditions with high levels of fertilizer, we arrive at 30–40% less yield in the older varieties (HAGBERG and MUNCK 1971) because of extreme lodging (Table 1). As pointed out by DE WIT (1968), it is rather futile to partition between the plant breeder's role and the role of human manipulation of the environment. Development is, of course, a combination of both. Maximal total yield potential (straw + seed) in the northern European barleys has not changed very much during the 20th century; but the percentage of seeds to straw has (HOLM 1970). The input of fertilizer increased total yield, but favoured vegetative growth. The main role of plant breeders has been to restore the ratio of the seed yield to total yield at steadily increasing levels of nitrogen fertilizer and at the same time increase crop reliability (winter hardiness, disease resistance, etc.).

Inspired by the studies in the 19th century of operational analysis of plant yield as related to soil fertility and through the concept of the blending characters of heritable variation, as advanced in Darwin's pangenesis theory, the handling of continuous variation patterns of different characters gradually developed into the science of biometry. The *discontinuous* quantum variation theory of heredity of Mendel came,

Table 1. Comparison between old and modern barley varieties grown at 60–90 kg nitrogen/ha (1965–1970, 10 trials)

Variety	Marketed in	Yield 100 kg/ha.	Relative yield
Gull	1913	44	100
Ymer	1945	51	116
Ingrid	1958	54	122
Hellas	1969	56	128
Kristina	1970–71	58	131

through the rise of "Mendelism" in the first decade of the 20th century, to stand in opposition to the concept of continuous variation of the biometric school (see review by DUNN 1965). This apparent conflict was bridged by JOHANSEN (1909), who made the fundamental distinction between genotype and phenotype, and by NILSSON-EHLE (1909), who demonstrated that several genes could influence one character, giving rise to a multitude of dose levels producing an almost continuous variation (polymeric inheritance). FISHER (1918) developed the statistical methodology for a rapprochement between the two approaches to variation, greatly promoting the concept of the "statistical gene" (MORGAN 1926) and the development of population genetics. Another line of development regarding gene structure and function can be drawn from BRIDGES' (1916) proof of the chromosome theory of heredity, MULLER's (1927) demonstration of artificial transmutation, the one gene — one enzyme hypothesis (BEADLE 1939), the confirmation of the deoxyribonucleic-acid (DNA) nature of the gene (AVERY et al. 1944), and finally over several intermediate stages to the concept of the genetic code-base triplets of DNA coding for specific amino acids—as the ultimate heritable quantum unit (NIRENBERG 1969).

The exploration of the nature of heredity has been a great help to the plant breeder for understanding how to *increase variation* efficiently through cross-breeding, polyploidy and mutation. However, from the point of view of *selection*, the current, basic plant-breeding work has not changed very much from the state of trial and error. The plant physiologist's concept of the "ideal plant", as well as biometrical models of variation, has not succeeded in making a direct

major contribution to the practical selection for high yield. These approaches (e.g., STROY 1965; GRAFIUS 1965) have in this respect mainly imposed their influence in evaluating the selection results from plant breeding.

2. Advancing the concept of quality

A. Cereal chemistry

One of the most evident crossroads between chemistry and (animal) physiology is the classical work by OSBORNE and MENDEL (1914), which defined some of the most important amino acids that are limiting for growth and maintenance. OSBORNE, in the 1890's specialized in defining various seed proteins on the basis of their solubilities in different solvents and analysed their amino-acid composition with methods developed by FISHER (see review by OSBORNE 1924). Albumins, globulins, prolamines and glutelins were thus defined respectively as proteins soluble in water (after dialysis), salt solutions, ethyl alcohol (70–90%) and dilute alkalis. Whereas many of these fractions from different mono- and dicotyledonous seeds were poorly defined, some of them could be easily purified and made to crystallize, e.g., the globulin edestin from hemp. The prolamines — e.g., gliadin from wheat, zein from maize, hordein from barley and kafirin from sorghum — deserved, according to OSBORNE (1924 p. 26–27), a definite name “for it is one of the best characterized groups yet found in either plants or animals”. OSBORNE comments further: “They are nearly or wholly insoluble in water, but their salts with acids or alkalis dissolve freely therein. They yield much glutamic acid, proline and ammonia, small amounts of arginine and histidine and little or no lysine. Prolamines have been found in the seeds of all cereals with the exception from rice, but have never been found in the seeds of any other family of plants”.

The knowledge of the *variation* of the amino-acid composition of the seed proteins was deliberately used by OSBORNE and MENDEL (1914) to define the qualitative needs of certain amino acids for the rat. Gliadin could serve the needs for maintenance, whereas zein could not fulfil

this purpose. Growth could consequently not be obtained by these two proteins. By additions of small amounts of purified amino acids and other proteins with different amino-acid composition, such as edestin, OSBORNE and MENDEL (1914) demonstrated convincingly that lysine is the limiting amino acid for growth in gliadin diets, whereas tryptophane is limiting for maintenance in zein diets. Addition of lysine and tryptophane to zein promote uniform growth. Consequently, these amino acids were recognized as indispensable for growth and maintenance. OSBORNE and MENDEL (1914) list the approximate amino-acid patterns of gliadin, zein, and casein omitting isoleucine, methionine, and threonine, which were recognized later. When threonine, as the last missing link, was found by ROSE (1935), complete diets could be made from purified amino acids, and thus the complete set of essential amino acids for rat growth could be defined: lysine, tryptophane, methionine + cysteine, threonine, isoleucine, valine, phenylalanine, leucine, histidine, and arginine. The first four amino acids attract special attention because they are limiting in most cereal diets.

OSBORNE (1924) also recognized the very different amino-acid patterns in proteins derived from endosperm, embryo, and leaf of the cereals — embryo and leaf proteins being much closer to the needs of growing rats (except for sulphur amino acids) than endosperm proteins. These differences are illustrated in a recent analysis from barley endosperm, embryo (including scutellum), leaf and stem (Fig. 1).

Endosperm is distinctly superior with respect to the quantity of the non-essential amino acids glutamic acid and proline, as well as ammonia (mostly obtained from amides after hydrolysis). The non-essential amino acids glycine, alanine, aspartic acid and the essential lysine, threonine, valine and arginine are deficient in endosperm compared to embryo, leaf and stem patterns, which only show minor differences in most amino acids. The stem is rich in ammonia and glutamic acid and the embryo in arginine. The high content of arginine in the barley embryo is paralleled by the high level in dicotyledonous seed (MUNCK et al. 1972 b), the latter derived from an analogous morphological structure. The unique amino-acid composition of the cereal endosperm is thus due to a development of special storage proteins among which the prolamines especially differ

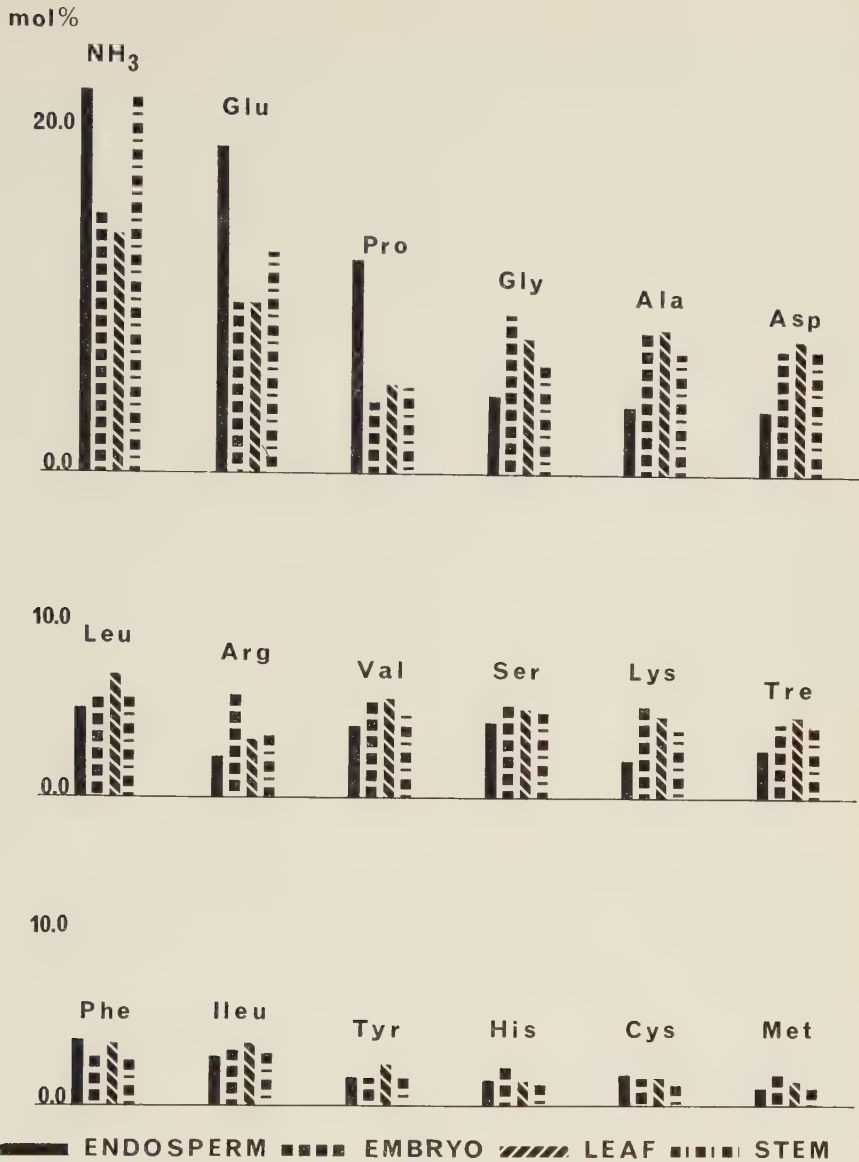


Fig. 1. Amino-acid composition (mol %) of a green barley plant displaying endosperm (milky stage), embryo, leaf and stem. NH₃ (ammonia), Glu (glutamic acid), Pro (proline), Gly (glycine), Ala (alanine), Asp (aspartic acid), Leu (leucine), Arg (arginine), Val (valine), Ser (serine), Lys (lysine), Tre (threonine), Phe (phenylalanine), Ileu (isoleucine), Tyr (tyrosine), His (histidine), Cys (cysteine), Met (methionine).

from the other Osborne fractionation groups. Additional discussions of cereal protein research are presented in Chapter IV.

In cereal analyses the principle of the Kjeldahl analysis for nitrogen is still widely used (Table 2) as a reference to other components, such as amino acids. The combustion method of DUMAS and CAHOURS (1842) gives a more complete determination of nitrogen, being, however, more elaborate. The values from nitrogen determinations are frequently in the literature multiplied with the conversion factor 6.25 for "Kjeldahl protein", which is used as a basis for calculating amino-acid composition in g per 16 g N (equal to g per 100 g Kjeldahl protein). Although criticized, this method of calculation is still most commonly reported. The true conversion factor may vary considerably in different proteins, and therefore other methods of calculation such as mmol amino acid per total mmol sum of amino acids (mol %) or mg amino acid per 100 mg of nitrogen should be considered. While the elementary analysis of nitrogen has practically remained unchanged for the last 90 years, amino-acid analysis has been greatly improved since the time of E. FISHER and OSBORNE. The ion exchange column separation technique of MOORE and STEIN with ninhydrin for identification is used in this paper according to the modification of EAKER (1970).

The essential amino-acid composition of cereals that can favourably be grown under southern Swedish conditions will now be considered (Table 2, MUNCK 1968, 1969). Eight varieties of (spring) barley were grown in the same year in Svalöf and Kalmar. There was a particularly low amount of precipitation in the latter location. Kjeldahl-protein content was 2.8 absolute percent higher in Kalmar, whereas most essential amino acids — especially lysine — were decreased in percentage of protein (g/16g N). Expressed in percentage of whole-seed, these amino acids, however, do increase which from the point of view of utilization is most important. The negative correlation, especially between crude protein and lysine (g/16 g N) owing to variety and environment, has been noted for most cereals except oats, and has been extensively discussed during the last 20 years (reviews by MUNCK 1964 a, MUNCK et al. 1971). The negative correlation is due to a greater relative synthesis of storage proteins low in lysine and other essential amino

acids at the higher levels of protein in the seed. When comparing the amino-acid patterns of, e.g., barley varieties, it is important to compare them on similar crude-protein levels. As will be demonstrated, deviations owing to environment, species, and variety exist that refute this apparent rule, which is treated as a natural principle by many scientists.

The essential amino-acid pattern in winter rye (Table 2) is very similar to barley except for leucine and phenylalanine, which are a little lower, whereas spring oats is significantly higher in lysine and especially in arginine, but lower in methionine than barley and rye. Oat protein is consequently potentially superior from the nutritional point of view. Spring wheat and winter wheat have nearly an identical amino-acid composition of proteins, being significantly lower as compared with barley protein in lysine, threonine, valine and phenylalanine. The artificially synthesized octoploid allopolyploid between wheat and rye — ryewheat or *Triticale* (winter form) developed by MÜNTZING (review 1962) — is very similar to wheat in amino-acid composition. This is natural because the six genomes (each with seven chromosomes) originating from wheat are epistatic over the two genomes contributed by rye. *Triticale* has, however, a significantly increased crude protein content in comparison with wheat, which greatly improves the nutritional quality of the whole seed. Because seed quality in some of the *Triticale* lines analysed is excellent and protein content is still high, the improved protein content is not due to shriveling. VILLEGAS et al. (1970) compared the lysine content of wheat, rye, and *Triticale* proteins and found an average improvement at 13.5% protein content of 12% in lysine in triticales when compared with hexaploid wheat varieties. These differences from the results in Table 2 are likely to be due to their use of hexaploid triticales, which have a higher ratio of rye to wheat genomes than the octoploids.

Comparing the amino-acid composition of protein from oats, barley, rye, and wheat with rice, maize, and sorghum (EGGUM 1968), rice was found to be similar to barley, except for the very low tryptophane content. The most important obstacle in rice-protein breeding is the low content of protein in this grain, down to 5% (JULIANO et al. 1968). Maize and sorghum, in relation to barley, are very low in lysine and

Table 2. The variation of important amino acids in barley, rye, triticale, wheat and oats

Cereal	No. of varieties	Protein	Gram amino acid per 16 g N								
			Lys	Arg	His	Met*	Tre	Val	Ileu	Leu	Phe
Spring barley	Sv 8	11.2	3.63	4.92	2.16	1.55	3.67	5.06	3.56	7.19	5.08
	K 8	10.2-12.1 14.0	3.4 4.1 3.33	4.5 5.5 4.62	1.9-2.4 2.19	1.4-1.7 1.47	3.4-3.7 3.53	4.7- 5.4 4.96	3.3-3.8 3.64	6.9 7.5 7.28	4.6-5.3 5.51
Spring barley		12.6- 15.9	2.9-3.6	4.3-4.9	1.9-2.7	1.4-1.6	3.3-3.8	4.8 5.1	3.5 3.7	7.1- 7.7	5.3 5.9
	Sv 7	10.8	3.72	5.03	2.30	1.57	3.58	4.88	3.56	6.47	4.82
Winter rye		9.6-12.4	3.3-4.0	4.6-5.4	2.1 2.4	1.4 1.7	3.4 3.7	4.6-5.1	3.5-3.8	6.1-6.9	4.6 4.9
	L 9	14.8	2.78	4.30	2.11	1.44	3.09	4.42	3.46	6.57	4.64
Winter triticale		13.5-16.1	2.6 3.2	4.0-4.7	1.9-2.4	1.2-1.6	2.9 3.3	4.2 4.7	3.2-3.6	6.3 6.9	4.4-4.9
	L 2	11.7	2.95	4.74	2.30	1.52	3.11	4.46	3.48	6.83	4.41
Winter wheat	Sv 5	13.1	2.85	4.67	2.28	1.57	3.10	4.53	3.61	6.90	4.61
		11.1 17.2	2.5 3.0	4.4 5.1	2.2-2.4	1.5-1.7	3.0-3.2	4.4-4.7	3.4- 3.9	6.7-7.3	4.4-5.6
Spring wheat	Sv 6	12.9	2.87	4.66	2.24	1.49	3.15	4.42	3.55	6.85	4.60
		10.5 15.3	2.6 3.2	4.4-5.1	2.1-2.4	1.3-1.7	2.8-3.3	4.0-4.7	3.6-3.7	6.4-7.1	4.4-4.8
Spring oats	Sv 9	11.0	4.03	6.31	2.25	1.38	3.64	5.00	3.79	7.27	4.90
		10.4 12.2	3.7 4.3	5.9-6.5	2.0-2.5	1.2-1.6	3.4 3.9	4.7-5.3	3.5-4.1	7.0-7.5	4.5-5.3

* Analysed as methionine without oxidation

Cereals grown in 1964

Precipitation in Kalmar very low

Sv = Svalöf (southern Sweden)

L = Lund (southern Sweden)

K = Kalmar (south-eastern Sweden)

Table 3. Comparison between cereals: falling number, crude-fibre and mineral composition

	n	Falling number (min)	% of dry matter				
			Crude fibre	Ash	Ca · 10 ⁻³	P · 10 ⁻²	K · 10 ⁻²
Barley	16	484 ± 54	4.6 ± 0.3	2.3 ± 0.3	43 ± 7	33 ± 5	54 ± 7
Rye	13	154 ± 70	2.5 ± 0.3	1.9 ± 0.1	40 ± 5	37 ± 5	59 ± 4
Triticale	12	65 ± 7	3.1 ± 0.3	2.0 ± 0.2	44 ± 6	40 ± 4	53 ± 4
Wheat	11	303 ± 123	2.7 ± 0.2	1.7 ± 0.1	40 ± 7	33 ± 3	48 ± 1
Oats	9	208 ± 97	10.3 ± 0.8	3.1 ± 0.2	80 ± 7	38 ± 3	54 ± 5

tryptophane and characteristically very high in leucine, the latter causing special balance problems from the nutritional point of view. Maize and sorghum proteins are thus even less balanced than wheat in nutrition. The new mutant genes with favourably changed amino-acid composition in maize (MERTZ et al. 1964; NELSON et al. 1965; McWHIRTER 1971; GLOVER et al. 1972) and in barley (MUNCK et al. 1970; DOLL 1971, INGVERSEN et al. 1972) will be discussed in Chapters III, IV and V.

The analytical procedures with regard to cereal carbohydrates in nutrition have aimed at fractionating the carbohydrates according to their physiological availability (see review by VAN SOEST 1966). The Weende crude-fibre analysis (cit. VAN SOEST 1966) was developed to provide an estimate of the unavailable components of the feed by extracting the presumed available part with strong acids and alkalis (Table 3). This method is widely used in conventional feed analyses in spite of its limitations. The sum of the other carbohydrates is represented as "the extractive residue" after subtracting crude fibre, ash and crude protein from total dry matter. The necessity of developing a fractionation method that is more appropriate from the physiological point of view has been pointed out by, e.g., VAN SOEST (1966) and SOUTHGATE (1969). The cell-wall complexes built up by hemicelluloses, pectin, and lignin are separated from starch by enzymatic extraction methods. Further fractionation is obtained by the use of weak acids, alkalis, and detergents. The presence of lignin is especially deleterious to the availability of carbohydrates and protein in all animals.

It can be seen from Table 3 that oats and barley

are particularly high in crude fibre as a result of the attached awns. In oats the awn parts constitute about 25 % of the seed, the main part consisting of about 50 % hemicelluloses, which are extractable in the conventional crudefibre analysis.

The content of mineral matter (ash) in cereals is related to the amount of cell-wall substances and crude fibre. Calcium is low, whereas phosphorus and potassium are more abundant in the seed (Table 3). Oats have a comparatively high calcium content. The main storage of phosphorus in the seed is in the form of phytic acids (inositol-hexaphosphates), which unfortunately have a tendency to react with calcium, magnesium, and iron to render them unavailable for animal digestion. The composition of phytic acids varies in different cereals, in oats being more effective in precipitating calcium than in wheat, suggesting that this may be one reason for the observation that oats are more rachitogenic than wheat (ASHTON and EVANS 1962).

Oats are rich in fat and contain about 5 % fat in the dry matter (Soxhlet ethyl ether extraction) compared with 1.5–1.7 % for *Triticale*, rye, wheat, and barley (Table 4). Cereals fats have a high content of unsaturated fatty acids.

When comparing the fatty-acid composition of crude fat, especially oats and rye deviated from the other cereals analysed (Table 4). Oats have a very high content of oleic acid (18:1), whereas linoleic acid (18:2) and particularly linolenic acid (18:3) are low. Rye is especially high in the last-mentioned highly unsaturated fatty acid, which might cause problems, because this acid is very sensitive to oxidation. The *Triticale* fatty-acid pattern resembles wheat more than rye, which is in agreement with the conclusions from their amino-acid composition

Table 4. Comparison between cereals: fat and fatty-acid composition

	n	Crude-fat (%) dry matter)	Fatty-acid composition of crude fat (%)					
			16:0	18:0	18:1	18:2	18:3	20:1
Barley	16	1.7 ± 0.1	22.5 ± 1.3	1.0 ± 0.2	13.9 ± 1.1	54.8 ± 1.8	6.8 ± 0.4	1.0 ± 0.1
Rye	13	1.5 ± 0.2	16.5 ± 1.2	0.6 ± 0.1	15.6 ± 1.6	55.6 ± 2.3	10.4 ± 1.7	1.3 ± 0.3
Triticale	12	1.5 ± 0.2	18.7 ± 1.5	0.7 ± 0.2	13.4 ± 2.4	60.1 ± 1.5	6.3 ± 1.4	0.9 ± 0.1
Wheat	11	1.6 ± 0.2	17.8 ± 1.0	0.6 ± 0.1	14.5 ± 1.5	60.5 ± 1.4	5.7 ± 1.0	0.9 ± 0.2
Oats	9	5.2 ± 0.4	16.3 ± 0.8	1.0 ± 0.2	40.2 ± 1.3	40.1 ± 0.9	1.7 ± 0.3	0.8 ± 0.1

(Table 2). The analysis presented in Table 4 for barley, rye, wheat, and oats accords with the study by LINDBERG et al. (1964).

Cereals are important sources of vitamins, such as thiamine, nicotinic acid, riboflavin, pyridoxine, pantothenic acid, and tocopherol. These vitamins are mainly present in the embryo and aleurone layer of the seed. Rye and oats are low and barley is high in nicotinic acid (HELLSTRÖM 1965).

In the seed, substances deleterious for animals can be produced either by the plant itself or by fungal infection. VIRTANEN (1964) reviews certain inhibitors (benzoxazolinones) to snow mold (*Fusarium nivale*) that are abundant in rye and wheat endosperms. Their physiological effects on animals are not fully known (MUNCK 1965). Especially rye is rich in certain phenols, 5-alkylresorcinols (WIERINGA 1967), which could serve a similar purpose from the plant's point of view but which, on the other hand, are found to be toxic for animals. According to WIERINGA (1967), rye seeds contain as much as 0.5% alkylresorcinols. In Table 5, the relative amounts of alkylresorcinols in rye, wheat and *Triticale* from samples mentioned in Table 2 are presented. Rye contains more than twice as much of these substances as wheat, whereas *Triticale* is, as expected, intermediate but more comparable to wheat than to rye in this respect. Cereals also contain tryptic inhibitors (LAPORTE and TRÉMO-LIERES 1962; SHYAMALA and LYMAN 1964), but at a lower level than in the soy bean. The adverse effect of tryptic inhibitors could be counteracted by heating.

Functional factors, which play a great role in food making, such as baking quality of wheat, have always been very important selection criteria in the development of cereals. The industrialization of these techniques by the milling,

Table 5. Alkylresorcinols in rye, wheat and triticale

From Table 2

Cereal	Number of varieties	Alcylresorcinols (units)
Rye	15	161
Range		129—192
Wheat	18	69
Range		56—79
Triticale	19	97
Range		78—124

baking and brewing industries has greatly stimulated research in food chemistry in the respective cereals. This is exemplified by the compilation of COOK (1962) on research in barley and malt. The "falling-number method" (Table 3) used as a screening method for baking quality (OLERED 1967) illustrates the need for rapid analysis in plant breeding and quality control. This method measures the viscosity of a meal suspension in a test-tube under gelatinization of the starch by heat on the basis of the time required for a body to fall through the suspension. *Triticale* (Table 3) and rye in comparison to wheat have especially low values owing to high alpha-amylase contents, which negatively affect baking quality.

In rice the amylase to amylopectin ratio of starch is important for cooking quality, a low ratio resulting in a sticky rice after cooking (JULIANO 1972). The taste varies widely in this respect in Asia.

B. Nutrition

As stated by VOGT (1970), several physiologists like QUESNAY (in 1785) were pioneering scientists

in economics. The investment of food in, e.g., an animal and its manifestations in growth, work, and excretion products are a suitable model to visualize the dynamics of an open economic system. Organisms such as man, pig, cow, and chicken have apparently different capabilities to utilize cereals. The efficiency of nitrogen is 27% for milk, 22% for poultry and eggs, 16% for pork, and only 6% for beef production (BREIREM and EKERN 1968). The micro-organism flora of the rumen permits digestion of cellulose and hemicellulose feeds, such as oat awns, barley straw, etc., and utilization of simple nitrogen substances like urea. The introduction of high-energy feeds, e.g., cereals, changes the production of organic acids in the rumen towards less acetic acid and more propionic acid, which seems to stimulate body-fat synthesis and cause a decrease of fat in milk (BREIREM and EKERN 1968). Thus for milk production, there is an optimum in energy concentration, whereas beef cattle could be successfully reared on more concentrated cereal diets.

The digestive system of the bird has developed quite a different specialization. In the upper part, there are no amylase-secreting glands, and the water content in the feed under treatment is comparatively low. Poultry are therefore — in highly viscous feeds, e.g., composed of rye or barley — dependent on enzymes that decrease viscosity and which are present in the cereal itself (Chapter V).

In man, rye bread is retained a longer time in the stomach than wheat bread (GLATZEL 1966), presumably because of viscosity effects.

The digestibility of crude protein varies greatly between different cereals. In rats, true digestibility figures for nitrogen are 100 for rice, 92 for sorghum, 90 for wheat, 88 for maize, 82 for barley, and 77% for rye (EGGUM 1968). The true digestibility of the important essential amino acids lysine and methionine are commonly lower in cereals (70–75%) as compared with serine and glutamic acid (90–100%) (EGGUM 1970 b). Threonine in rice (MIYAZAKI 1969) also seems to have an impaired digestibility in spite of high total availability for crude protein.

The absorption of amino acids is transmitted roughly through four transport mechanisms: (a) most neutral amino acids, (b) dibasic, (c) dicarboxylic, and (d) imino acids + glycine (SAUNDERS and ISSELBACHER 1966). These prin-

ciples seem to be inherent not only for the transport of amino acids from the gut to the portal vein but also more generally, e.g., from the blood through any cell membrane. There is a sharp antagonism between, e.g., leucine and isoleucine, and lysine and arginine. The overall effect on the organism of such amino-acid imbalances is impaired eating and growth (HARPER 1966). In maize and sorghum the extremely high content of leucine depresses the utilization of isoleucine, increasing the needs of this amino acid (BENTON et al. 1955). An excess of methionine in supplementation, which strongly depresses growth, is a further example of amino-acid imbalance. The simplified concept of the first, second, etc. limiting amino acids, as well as essential and non-essential amino acids, is thus an oversimplification, but occasionally suitable in practice. Interactions in nitrogen metabolism between amino acids as well as between amino acids and other nutrients, viz. the vitamins B₆ and niacin (RUBIN 1966), must be considered.

The energy balance of an organism is another aspect of the metabolism. The metabolized energy could be measured calorimetrically and is the difference between the energy of the feed on the one hand and the energy in the faeces, gases, and urine on the other (ERIKSSON 1966). Only a small part of the absorbed energy is stored in the form of chemical bonds in the growing organism. This is the net energy for production, which in poultry comprises about 30% of the metabolized energy (JACOBSEN et al. 1960). The rest of the energy is used for muscular activity and for heat production — the so-called heat increment. Other factors in the diet, such as the amino-acid balance or toxins from fungi, like puromycin, actinomycin, and aflatoxin, affect RNA and protein synthesis. In such extreme and negative diets, the metabolized energy is, to a very high degree, absorbed in the heat increment.

Nutritional studies in man are naturally very difficult to carry out. Nitrogen balance and energy investigations require often hospitalization for long periods. Nutritional problems in humans are therefore most frequently studied when they appear in society. The problems of overnutrition in certain countries like Sweden (BJURULF and LINDGREN 1964), resulting in increased morbidity and mortality, definitely indicate that the optimal goal is not rapid growth and "fattening" as it is in animal husbandry. On the other hand, the

serious effects of inadequate nutrition owing to lack of calories, protein, vitamins and minerals, especially in infancy, (EICHENWALD and FRY 1969) often result in an impairment of mental functions and in a decreased capacity in work and organization. The problems are immense as regards stating the optimal composition of foods because of the variation in human requirements (WATERLOW 1970), and likewise as regards the evaluation of the dietary intake in practice (HEGSTED 1969). FAO and WHO (1965) have made such an effort in their recommendations in the report *Protein Requirements*.

There is an obvious need for representative animal experimental procedures that could be used on a large scale in studying nutritional problems, both concerning human aspects and animal husbandry aspects. With respect to protein metabolism, a large number of such techniques have been developed. One of the most simple methods is PER (protein efficiency ratio) determination introduced by OSBORNE and MENDEL (1919). Growth (g) is recorded in relation to crude protein intake (g) as expressed in the PER ratio. THOMAS in 1909 (cit. EGGUM 1970 b) introduced the more elaborate nitrogen balance studies in rats, which were refined by MITCHELL in 1924. With this method, the biological value of a protein is defined as the percentage of the actually digested nitrogen that is retained in the body. Corrections are made for losses due to endogenous nitrogen (from tissues in the intestines) and due to faecal and urinary nitrogen. BENDER and MILLER in 1953 modified the nitrogen balance method, measuring nitrogen content in the carcass and subtracting the nitrogen carcass content of an o-group fed a proteinfree diet (to compensate for endogenous nitrogen). The difference was used in calculating the nitrogen retention, or the net protein utilization (NPU). EGGUM (1970 b) records, in nitrogen balance studies (protein at 9.4%) with growing restrictively fed rats, the biological value and true digestibility of protein by analysing faeces and urine uncontaminated by food. This method also facilitates true digestibility studies of single amino acids in cereals. The results from rats and swine reflecting true digestibility were comparable. It is an obvious advantage to record all of these parameters, especially when studying cereal proteins where availability is an important issue. I have used mice in large-scale studies of growth

in protein and fat as well as nitrogen retention through carcass analysis (MUNCK 1964 b, 1970 b).

In this paper, studies of mice will be used to exemplify some nutritional problems in cereals. The method has changed during the 10 years of research. Originally, group feeding with 2–4 animals per cage was practised, but later (1969) single feeding was introduced. The strains have been males from CBA, Swiss $\times$ CBA, and SPF/NMRI. The first two strains are the ones that are best controlled from the genetic point of view, bred in the laboratory, whereas the latter is a commercial specific pathogen-free (SPF) strain. The influence of the strain in an experiment regarding protein quality is discussed by MUNCK (1970 b). The feeding period changes from 20 days in CBA to 14 days in Swiss $\times$ CBA and 11 days for the SPF/NMRI strain, giving approximately the same growth response. The hybrid and the specific pathogen-free strains have a considerably greater growth rate. Minerals and vitamins are supplemented according to MUNCK (1964 b) and JOSEFSSON and MUNCK (1972). Vitamins are dissolved in 20 ml of water and incorporated in a small portion of the diet (about 50–100 g), which is dried at 40°C. This mixture, as well as the minerals, is thoroughly mixed with the dry diet. It is very important not to treat a cereal meal with water when preparing diets because of the rapid changes due to enzymatic effects (MUNCK 1964 c).

The vole (*Microtus pennsylvanicus*) has been used by ELLIOT (1963) and SHENK et al. (1970) in screening studies especially involving grasses. This animal has an optimum performance at fibre levels between 8 and 23%, whereas mice and rats react negatively at such high fibre levels.

Another important approach is the analysis of the dynamic fluctuation of amino-acid content in blood plasma, a method which could be used for, e.g., rats and humans (KIHLEBERG 1970). The free amino-acid pattern in blood after a meal reflects reasonably well amino-acid absorption. A similar method using urea concentration (BERGNER et al. 1970) demonstrated a strong negative correlation between urea content in the plasma and the biological value for protein in rats.

Insects, such as the confused flour beetle (*Tribolium confusum*) and a similar species, *T. castanum*, have been proposed for screening purposes by LOSCHIAVO et al. (1969) in barley and by DE MUELENAERE and QUICKE (1960) in maize. A more nutritious cereal is characterized by a shorter larval period.

The ciliate *Tetrahymena pyriformis* has been used by many scientists for screening, e.g., STOTT et al. (1963). This micro-organism can take up and digest small particles in its unicellular body.

It can also digest them in the medium by exoenzymes. The rate of growth is determined by cell counts. According to RÖLLE and EGGUM (see EGGUM 1970 b), there is a good relative agreement between rats and *Tetrahymena* cellcounts on diets that are not limiting in their content of sulphur amino acids, whereas there was no correlation on other diets. This indicates that *Tetrahymena pyriformis* has a lower relative requirement for sulphur amino acids than rats. Other microbiological methods for studies of the individual availability of important amino acids have been developed by FORD (1962, 1964, 1965).

Enzymatic methods involving the release of amino acids after digestion with different enzymes, such as pepsin and trypsin, have been extensively studied by SHEFFNER (1967) and MAURON (1970), as estimations of amino-acid availability, with applications in the study of processing effects.

a. Comparing swine, mice and rats fed diets of different protein quality

In Denmark, feed utilization and the production of lean carcasses are important factors in the swine-rearing economy (CLAUSEN 1963). Barley is used as a suitable cereal together with protein additives to reach this aim in a moderate, restrictive feeding programme. MADSEN et al. (1970) studied the protein and amino-acid supplementation in barley diets for swine. These diets were also fed to mice and rats and will be discussed here (Tables 6 and 7). The diets were composed of barley (diet No. 5), with lysine and methionine supplementation (4), barley + gluten (3), barley plus gluten with lysine and methionine supplementation (2), and finally a barley-skim-milk diet (1). The last-mentioned diet is regarded to be an ideal one for swine. Wheat gluten was added to barley to obtain approximately the same daily intake of digestible protein for swine on diets (2) and (3) as with the skim-milk diet (1). Lysine and methionine were added to barley (4) to obtain the same daily intake of these amino acids as in the unsupplemented gluten-barley diet (3).

These diets were also fed ad libitum to mice that were analysed with respect to carcass composition (Table 6). The skim-milk diet (1) and the supplemented gluten diet (2) were superior with respect to gain in crude protein for mice

Table 6. Mouse feeding experiment with different protein qualities. Diets from MADSEN et al. 1970.

Diet	Dietary crude protein dry matter (%)	Gain per animal		Consumed per animal			PER*	NR**
		Total weight (g)	Dry matter (g)	Fat (g)	Crude protein (g)	Dry matter (g)		
1. Barley + skim milk powder	17.0	13.3 ± 1.2	4.36 ± 0.31	0.90 ± 0.20	2.66 ± 0.11	70.7 ± 3.1	1.10 ± 0.07	22.1 ± 1.1
2. Barley + gluten + lys + met	16.1	13.1 ± 1.0	4.35 ± 0.31	0.78 ± 0.26	2.64 ± 0.15	68.7 ± 1.7	1.18 ± 0.06	23.9 ± 0.9
3. Barley + gluten	16.0	12.3 ± 0.8	4.17 ± 0.28	0.95 ± 0.28	2.43 ± 0.08	69.1 ± 5.3	1.11 ± 0.04	22.0 ± 0.9
4. Barley + lys + met	10.9	11.2 ± 0.4	3.59 ± 0.11	0.73 ± 0.10	2.14 ± 0.12	74.6 ± 3.3	1.37 ± 0.04	26.3 ± 1.3
5. Barley	10.4	10.9 ± 1.1	3.47 ± 0.59	0.80 ± 0.27	2.07 ± 0.26	73.8 ± 5.6	1.42 ± 0.04	26.8 ± 1.5

2 CBA males per cage in 4 cages per diet, 20-day feeding trials

* g gain per g crude protein (N × 6.25) consumed

** Nitrogen retention

when compared with the low-protein diets (4) and (5). The unsupplemented gluten diet was intermediate to these extreme groups. Nitrogen retention (NR) is, for physiological reasons, lower on high-protein diets than on low ones even with regard to identical proteins. There is no effect of amino-acid supplementation on NR in the barley diet (5-4) and only a small response in the gluten diet (3-2).

The mouse feeding results presented in Table 6 are directly compared with the swine results in Table 7 (MADSEN et al. 1970). The relative consumption figures for protein, lysine and methionine compared with the skim-milk diet set equal to 100 are comparable for the two species. Carcass gain (fat-protein estimates) and the feed consumption (feed units/kg gain) are also given. Feed consumption as related to gain is the parameter that gives the best comparison. It is obvious that mice have a much lower lysine requirement for growth than swine. Mice can utilize the unsupplemented gluten diet (3) better than the supplemented barley diet (4), although the relative lysine intake is similar in the two diets (3-4). In the swine experiment both these diets result in very poor growth. Lysine plus methionine do not appear to be limiting in the pure barley diet (5-4) for mice, whereas there is a marked effect from supplementation for the swine. The effect of amino-acid supplementation to the gluten diet (3-2) for the mice is much smaller than for the swine. While the difference between the two extreme diets (1-5) in feed units/kg gain is +27% for the mice, the figure is +127% for swine. For the mice an amino-acid supplementation (5-4, 3-2) tends to give less fat as compared to protein while, this effect is much more significant for the swine in the dietary comparison (3-2). The skim-milk diet gives the optimal carcass composition in swine. The positive supplementation effects on carcass composition are in accord with results from mice (MUNCK 1966 b) and swine (CLAUSEN 1963).

In studying protein quality in trials with laboratory animals, the crude-protein content is usually fixed at 10%, obtained by dilution with starch. This method could be discussed from the practical point of view because the relative needs of the different amino acids could be changed with altered protein levels. The diets in Tables 6 and 7 were also fed to rats by EGGUM in restrictive feedings at a 9.4% level (MADSEN et al. 1970).

The biological value of barley (72.7%) is not increased with the amino-acid supplement (73.2%), which is in agreement with the mouse trial. There is a marked increase in the biological value (BV), from 59.3 to 67.1% owing to supplementation of the barley-gluten diet. The net protein utilization (NPU) figures for rat give a similar picture. The barley-skim-milk diet obtains a BV of 90.0%. These responses in rats are higher than in the mouse experiment. On the other hand, it is obvious that the difference between rats and mice should be considerably less if the rats were given an approximately 60% higher crude-protein intake for diets 1, 2, and 3 to be comparable to the mouse-feeding conditions. *Thus both mice and rats in the barley supplement experiment described are much less dependent on certain limiting amino acids, among them explicitly lysine, than swine.*

b. *Comparing the nutritive quality of barley, rye, wheat and Triticale fed to mice*

The cereals analysed in Tables 2-5 will be considered in toto, as they are fed to CBA mice after milling and supplementation with vitamins and minerals (MUNCK 1968, 1969). In Table 8, the average feeding results with standard deviations from diets of barley, rye, *Triticale* and wheat from Table 2 are discussed (1-4) and compared with the average figures of some low-protein lines (5-8) included in the original material (1-4). The CBA male mice were unable to grow on whole oats because of its high-fibre content. Average food intake in dry matter is quite constant when comparing the cereal species in the whole material (50.8-52.0 g). Crude-protein intake is therefore dependent on the dietary protein content.

As to amino-acid composition (Table 2), rye and barley are almost identical, with a higher level of essential amino acids (e.g., lysine, methionine, threonine) than wheat and *Triticale* — the latter two also having a similar amino-acid composition. When comparing the feeding performance of barley (5) and rye (2) at similar protein content, it is seen (Table 8) that gain in dry matter, gain in protein, and especially gain in fat are less for the animals receiving the rye diets in spite of similar protein intake and amino-acid composition. A comparison of rye (2) and wheat

Table 7. Comparison between pigs and mice fed the same diets

Diet	Pig trial, restrictive to 90 kg (MADSEN et al. 1970)							Mouse trial, 20 days ad libitum (Table 6)						
	Consumed per day							Consumed			Carcass			
	Days 20 to 90 (kg)	Digest. protein (g)	Lys (g)	Met (g)	Daily gain (g)	Feed (units per kg gain)	Fat area (cm ²)	Crude protein (g)	Lys (g)	Met (g)	Gain (g)	Feed (units per kg gain)	Gain protein (g)	Total Fat Prot.
1. Barley + skim milk Standard	95 100	231 100	16.1 100	5.9 100	722 100	2.62 100	25.5 100	12.1 100	1.67 100	0.26 100	13.3 100	5.32 100	2.66 100	42 100
2. Barley + gluten + lys + met Relative to diet 1	110	98	96	97	95	103	111	92	82	92	98	98	99	95
3. Barley + gluten Relative to diet 1	174	90	48	85	55	166	143	92	55	85	92	106	91	107
4. Barley + lys + met Relative to diet 1	195	57	51	88	59	164	155	68	55	73	84	125	80	100
5. Barley Relative to diet 1	274	52	40	58	39	227	153	64	48	62	82	127	78	110

Table 8. Nutritive values of barley, rye, triticale and wheat for mice

Diet	Sam- ples (no.)	Cages (no.)	Dietary crude protein dry mat- ter (%)	Gain per animal			Consumed per animal			PER	NR
				Total weight (g)	Dry matter (g)	Fat (g)	Crude protein (g)	Dry matter (g)	Crude protein (g)		
<i>Whole material</i>											
1. Barley	16	35	12.6	9.2 ± 0.7	2.97 ± 0.24	0.85 ± 0.11	1.72 ± 0.17	51.2 ± 3.0	6.4 ± 0.9	1.45 ± 0.12	27.0 ± 2.0
2. Rye	11	19	10.8	7.8 ± 1.1	2.18 ± 0.14	0.42 ± 0.14	1.39 ± 0.25	51.0 ± 3.8	5.5 ± 0.8	1.41 ± 0.11	25.1 ± 2.0
3. Triticale	12	24	14.8	9.7 ± 1.0	2.98 ± 0.30	0.75 ± 0.10	1.77 ± 0.20	50.8 ± 3.9	7.8 ± 0.9	1.25 ± 0.08	22.7 ± 1.4
4. Wheat	11	22	13.0	8.6 ± 1.0	2.72 ± 0.34	0.76 ± 0.15	1.57 ± 0.18	52.0 ± 3.4	6.8 ± 1.3	1.29 ± 0.14	23.4 ± 0.5
<i>Low-protein lines</i>											
5. Barley	8	18	11.1	8.7 ± 0.5	2.82 ± 0.16	0.84 ± 0.09	1.61 ± 0.11	51.7 ± 3.7	5.8 ± 0.4	1.51 ± 0.08	27.9 ± 1.5
6. Rye	3	7	9.6	6.9 ± 0.8	1.88 ± 0.37	0.36 ± 0.18	1.18 ± 0.17	49.0 ± 3.0	4.7 ± 0.4	1.44 ± 0.12	24.9 ± 2.3
7. Triticale	3	6	13.9	9.3 ± 0.5	2.92 ± 0.14	0.78 ± 0.10	1.70 ± 0.07	51.0 ± 2.3	7.1 ± 0.3	1.32 ± 0.06	24.2 ± 0.6
8. Wheat	3	6	11.2	8.2 ± 0.9	2.58 ± 0.29	0.72 ± 0.17	1.47 ± 0.17	52.1 ± 4.1	5.8 ± 0.7	1.41 ± 0.12	25.3 ± 1.1

4 CBA mice per cage, 20 day experiment

(8) shows that rye is inferior also to wheat, especially regarding gain in fat. Barley is superior in nitrogen retention when compared to wheat (1–4), whereas the barley-rye differences are smaller. *Triticale* confers a good growth rate because of its high protein content and has a performance like high-protein wheats. Feeding results on rye diets seem to be more affected by dietary protein reduction than any of the other cereal diets compared. In order to verify the exclusive effects of rye diets, rye and wheat were fed in a supplementation experiment (Table 9). The original crude-protein levels of the unsupplemented diets (1–2) were comparatively high (13.7–14.5%). Lysine (3–4) and lysine+egg albumin (5–6) were added to compensate for differences in the most important essential amino acids revealed by amino-acid analysis of the two cereals. Lysine supplementation increases gain parameters and NR in both cereals, whereas a further addition of egg albumin has no effect. On the unsupplemented diets, intake of dry matter and crude protein is in this case considerably lower with the rye diet. The depression is mostly eliminated with supplementation. On the three levels of comparison, gain in dry matter, gain in crude protein, and gain in fat are lower in rye than in wheat. Nitrogen retention seems to be slightly higher and food intake lower in rye than in wheat. *The two experiments (Tables 8 and 9), included for orientation, indicate that the deviation in feeding performance for rye compared with, e.g., wheat or barley could not completely be explained by differences in protein quantity or amino-acid composition.*

Feeding high amounts of rye to animals has for a long time been considered disadvantageous. KNIERIEM (1900) found negative effects from rye compared with barley for cow, horse, pig, hen and rabbit. In the literature, there seems to be a general agreement of the unsuitable effects of feeding rye, especially to young birds, whereas high levels of rye (20–50%) could occasionally be used for fattening swine (RICHTER et al. 1961). In feeding trials with chickens (HALPIN et al. 1936), more than 15% rye in the diet caused wet droppings of faeces, decreased feed consumption and growth. Mortality is considerably increased. Similar effects have been recorded when feeding barley that has been grown under dry climatic conditions (WILLINGHAM et al. 1960). BURNETT (1966) indicated that this was due to

the high viscosity and the low enzyme content, e.g., alpha-amylase in barley from such areas. As rye also is very high in viscosity (MUNCK 1968), this factor may contribute to the unfavourable feeding results in chicken. However, viscosity effects are likely to be minimal in Europe because of the high enzyme content of rye in this climate.

The negative effect of high viscosity seems, however, to be a rather special problem for bird feeding. In mouse trials at Svalöf, we have found that the faeces is normal and that the food intake and water consumption are unaffected when feeding barley grown in the western dry zone of the U.S.A. as compared with the same varieties grown in the more humid climate of Sweden (unpubl.). BURNETT and NIEL (1964) obtained no difference in feeding barley with and without enzyme supplementation to swine.

HOLTZSCHUH and TRAUTMAN (1962) explained that the negative effect of rye was due to its contamination with poisonous weed seeds or ergot (*Claviceps purpurea*). WIERINGA (1967) found, however, that the phenols – 5-n-pentadekylresorcinols, which occur normally in rye and wheat – are deleterious to rats and swine fed purified resorcinols. Young animals were more sensitive than old ones, and a high protein content in the feed mixture evoked fewer symptoms – the latter observation of WIERINGA being in agreement with the mouse trial (Table 8). The depressed growth could not completely be explained by a decrease in food intake (WIERINGA 1967). The alkylresorcinols should contribute to the negative effects in mice fed rye in the experiments from Tables 8 and 9, whereas it is less likely for the viscosity to have a direct effect. Another important factor that affects the feeding value of rye is the low availability of protein, carbohydrates and fats in comparison with those from wheat and maize (JANICKI and KOWALCYK 1967). Barley is similar to rye in this respect (DAVIDSON et al. 1962).

From the cited examples, it is obvious that several nutritional factors besides those commonly expected in cereals could be indicated. On the other hand, it is very difficult to isolate these factors to study them “in a pure state”. After considering some views concerning the impact of environment (Chapter II) and the genetic variation (Chapters III and IV) on the cereal seed, I will return to selected extremes of variation that

Table 9. Comparing the nutritional performance of wheat and rye in a supplementation experiment compensating for differences in amino-acid composition of the diets

Diet	Dietary crude protein dry matter (%)	Gain per animal*		Consumed per animal				PER	NR
		Total weight (g)	Dry matter (g)	Fat (g)	Crude protein (g)	Dry matter (g)	Crude protein (g)		
<i>No supplementation</i>									
1. Wheat	14.5	10.5 ± 0.3	3.78 ± 0.06	1.17 ± 0.04	1.94 ± 0.08	63.4 ± 2.2	9.2 ± 0.3	1.14 ± 0.05	21.6 ± 1.0
2. Rye	13.7	9.4 ± 0.9	3.00 ± 0.50	0.74 ± 0.31	1.74 ± 0.28	53.7 ± 2.4	7.4 ± 0.3	1.26 ± 0.13	23.5 ± 3.9
<i>+ L-lysine HCl</i>									
3. Wheat (+ 3.2 g/kg)	14.7	11.9 ± 0.8	4.37 ± 0.39	1.37 ± 0.22	2.24 ± 0.16	62.5 ± 2.3	9.2 ± 0.3	1.29 ± 0.05	23.3 ± 1.1
4. Rye (+ 1.8 g/kg)	13.9	10.5 ± 0.2	3.64 ± 0.30	0.93 ± 0.13	2.10 ± 0.10	59.5 ± 1.4	8.3 ± 0.2	1.27 ± 0.03	25.3 ± 1.5
<i>+ L-lysine HCl and egg albumin</i>									
5. Wheat (+ 26 g/kg)	16.6	11.6 ± 0.8	4.13 ± 0.25	1.32 ± 0.12	2.24 ± 0.14	58.9 ± 1.8	9.8 ± 0.3	1.18 ± 0.04	22.5 ± 0.9
6. Rye (+ 24 g/kg)	15.9	10.9 ± 0.7	3.57 ± 0.39	0.89 ± 0.26	2.08 ± 0.23	56.7 ± 2.3	8.4 ± 0.3	1.30 ± 0.05	23.0 ± 1.8

* CBA male mice (2 × 4 per diet), 20-day experiment

could be used in feeding tests and in a discussion of factors of basic nutritional importance in cereals (Chapter V).

C. Screening analyses in the study of variation in protein quantity and quality

Biochemistry gained important knowledge in the 1930's and 1940's about the metabolic pathways by screening deviating micro-organisms with agar-plate techniques employing selective substrates. In plant breeding, it was not until the last decade that a general interest has arisen for efficient screening methods as regards protein quantity and quality in cereals. The emphasis seems, however, still to be placed on developing new analytical techniques, whereas the appropriate use of methods already existing for mass screening is attracting less attention.

There has been a discussion in literature regarding the advantages to apply, in plant breeding programmes, non-destructive analytical methods on a single seed basis leaving at least the embryo intact (Recommendations from the IAEA/FAO Symposium: *New Approaches to Breeding for Improved Plant Protein*, Vienna, 1969). With such techniques used in, e.g., a backcross programme in barley to incorporate a mutant gene in a suitable background, breeding could be effectively speeded up provided the character segregates in the spikes of a heterozygous plant. Protein (nitrogen) content has a complex genetic background and is very much dependent on environment. Crude-protein content varies greatly between seeds from the same plant, and the application of non-destructive, single-seed techniques for nitrogen determination in breeding programmes seems, therefore, to be doubtful. It was pointed out by MUNCK (1970 a) that in barley and maize segregating for the high-lysine genes the ratios between, e.g., lysine and arginine or lysine and nitrogen are much less influenced by environment than the single analysis lysine, arginine and nitrogen. The simultaneous analysis of two amino acids, two reactive groups, one amino acid and one chemical element, or two chemical elements (like N and S) could thus be more promising to consider in a non-destructive analysis. The double analysis, however, increases the analytical complication.

The Kjeldahl method for nitrogen determination undoubtedly commands, even in the 1970's,

a leading position as the general analysis of reference. The varied flora of screening analyses for nitrogen or "protein" content are thus regularly correlated with the Kjeldahl method to test its "suitability". Although the Dumas method (EBELING 1968), from the chemical point of view, provides a more complete determination of nitrogen, it has not supplanted the Kjeldahl method. More or less non-destructive physical methods, such as fast neutron activation analyses (DOTY et al. 1970; KOSTA et al. 1969), the N- γ -technique (NIEMANN and NEUMÜLLER 1972), the N-proton technique (JOHANSSON et al. 1969), as well as nuclear magnetic resonance (NMR) (HEDVIG and ZENTAI 1969) and electron spectroscopy for chemical analyses (SIEGBAHN 1967), are under rapid development (KLEIN and KRAMER 1972). Although some of these techniques, like neutron activation and NMR, have been used in screening programmes, they have, in general, not replaced the Kjeldahl method probably because of the expensive investment for equipment and lack of training.

In Svalöf, we have speeded up Kjeldahl analyses by using a test-tube rack (Tecator AB, Box 308, 25104 Helsingborg, Sweden), which permits 40 single micro-Kjeldahl samples to be digested in 20 minutes. After diluting to 70 ml, 0.5 ml of the digest is pipetted to a Technicon automatic nitrogen sampler, which utilizes a colorimetric technique for determining the ammonia in the digest. Forty samples are recorded in 1 hour. With this method, about 200 single analyses are made each day, the milling and weighing procedures being by far the most costly. Milling could, however, be speeded up greatly, up to 30–50 samples (5 g) per hour by using a Udy cyclone mill. The price per single Kjeldahl analysis with this method is about 3:50 Swedish Kronor (U.S. \$ 0.75).

A large number of chemical screening techniques for estimating crude-protein content have been developed to avoid the elaborate Kjeldahl method. These methods estimate specific reactive groups, the number of which is more or less correlated with total nitrogen according to Kjeldahl. The biuret method (PINCKNEY 1961) represents such a possibility, measuring the peptide bond by the binding of copper colorimetrically. Another possibility is to apply dye-binding methods, estimating the basic amino groups as outlined by FRAENKEL-CONRAT and COOPER (1944), ROSENBERG and KLOTZ (1960). UDY (1954, 1956) introduced the measurement of dye-binding capacity (DBC) in wheat to estimate crude-protein content and further revised and

extended the method to other cereals (review by UDY 1971).

The most suitable method uses the acid diazo dye Acilane Orange G, also called acid orange 12 (CI 15970), which is chemically the monosodium salt of 1-phenylazo-2-naphthol-6-sulfonic acid. At pH 2.6, the anionic sulfonic acid dye binds quantitatively with the cationic imidazol, guanidine and amino groups corresponding to histidine, arginine, and mainly to lysine in the proteins. The reaction is performed in a meal suspension and the dye precipitates the protein. A decolorization of the original solution is thus obtained, which can be measured colorimetrically in a short-path cuvette. Lowmolecular weight peptides, etc., are consequently not precipitated. Nonspecific binding can occur owing to, e.g., starch or the calcium ion. Since the binding is due to weak ionic forces, the DBC procedure is founded on a dynamic, partly reversible reaction with an equilibrium varying for different proteins.

DBC is also dependent of the milling procedure. The evaluation of the nature of DBC in a complex material as cereals is thus extremely intricate. Empirically, the method, according to Udy, has shown excellent correlations with Kjeldahl analysis *within* different materials, such as wheat, barley, oats, maize and soybean.

The DBC method has been approved as an official A.A.C.C. (American Association of Cereal Chemists) method (46-14), each crop having a special standardization curve related to crude protein according to Kjeldahl. Through these relations, DBC values are expressed in Kjeldahl ($N \times 6.25$) terms as "Udy Kjeldahl" or dye-binding protein (DBP) values. The method is valid provided there is a strong correlation between nitrogen and the different basic amino acids (BAA). This fact is seldom appreciated by workers using the DBC method for nitrogen.

In 1962, a heat-damaged barley sample, of cultivar Rika, obtained from MOSSBERG (Weibullsholm Plant Breeding Institute) was fed to mice in my laboratory and proved to be of low nutritional quality (MUNCK 1964 c). Growth could, however, be completely restored with lysine supplementation. This sample was very low in dye-binding as compared with its Kjeldahl protein content (MOSSBERG pers. comm.). MOSSBERG (1965) demonstrated that the ratio Kjeldahl protein (KP) to DBP can be changed in wheat by extraction, germination and heating. Further heat treatments in barley (MUNCK 1966 a) produced samples having a significant correlation between mice growth and DBP (MOSSBERG 1966) and between DBP and lysine (Fig. 43).

It was thus realized by MOSSBERG that the ratio DBP to KP could be used as an estimation of

lysine and BAA in the protein. This idea was tested in a cereal material, with variable amino-acid composition, consisting of barley, oats, rye, wheat, triticale (Table 2), and maize varieties (including opaque-2) (MUNCK 1968; MOSSBERG 1969, 1970). Through this study, it became obvious that the different DBC to KP relationships for the various cereals were due to their varying contents of basic amino acids. All samples from the six different cereals did fit into the same relation DBC—BAA. As lysine, histidine and arginine were positively correlated, the DBC to KP ratio could be used for screening lysine. This was tested in a survey of the World Barley Collection and resulted in the discovery of the high-lysine cultivar Hiproly (High-protein and lysine barley—CI 3947) (HAGBERG and KARLSSON 1969; MUNCK et al. 1969). The "declination" from the normal DBC—KP regression is deliberately used for estimating lysine. Thus, when high-lysine barley lines are introduced in the U.S.A., the A.A.C.C. method of DBC is an inaccurate method for Kjeldahl protein, and should be changed into a method to evaluate lysine (cf. MUNCK et al. 1971). In the work on barley protein in Svalöf, the DBC procedure of MOSSBERG (1969) is used for screening lysine. This method has some advantages and limitations worth discussing. As dye-binding is a dynamic equilibrium reaction, the ratio between the dye-protein complex and the free molecules of the dye is of considerable importance. This is illustrated in the barley material referred to in Fig. 2 originating from material in Table 32. DBC to KP is plotted against KP for samples analysed first at a constant sample weight (0.5 + 20 ml dye solution, circles) and secondly at a constant protein weight (60 mg + 20 ml dye solution, triangles). It is apparent that part of the negative slope coefficient DBC: KP—KP at a constant weight of the sample is dependent on the variable protein content per se. At low-protein contents in the material, 0.5 g samples result in a higher concentration of dye in the solution than higher protein contents. This affects the DBC to KP ratio, over-estimating BAA at low protein contents and underestimating it at high protein levels.

In Table 10, wheat (A), barley (B) and oats (C, D) are analysed for KP, BAA and DBC at 20, and 40, and 60 mg of crude protein. In wheat and barley protein, there is a significant decrease

140. mMol DBC / g protein

$$\bullet \quad y = -0.0470x + 17179 \quad r = 0.9768$$

$$\Delta \quad y = -0.0275x + 14676 \quad r = 0.9718$$

1.20

1.00

0.80

5

10

15

Crude protein %
20

Hiproly



Fig. 2. The relation between dye-binding (mMol/g protein) and crude protein (%) in normal lysine barley and Hiproly at a constant weight of 0.5 g samples (circles) and 60 mg crude protein ($N \times 6.25$, triangles) per 20 ml dye solution.

Table 10. Amino-acid composition and dye-binding capacity (DBC) of wheat, barley and oats

Samples	Crude-protein content (%)	Lys	His per 16 g N	Arg	BAA*	Dye-binding (μmol/mg) at a constant weight of protein of			
						20 mg	40 mg	60 mg	Diff. 20—60
A. Wheat									
695-40	12.6	2.74	2.34	4.64	9.72	1.22	1.14	1.09	0.13
695-25	14.2	2.45	2.17	4.61	9.23	1.11	1.09	1.04	0.07
695-13	16.5	2.34	2.14	4.19	8.67	1.03	1.02	0.97	0.05
695-8	17.9	2.32	2.15	4.03	8.50	0.98	0.99	0.95	0.03
695-19	19.8	2.33	2.17	4.13	8.63	0.98	1.01	0.97	0.01
Mean	16.2	2.44	2.19	4.32	8.95	1.06	1.05	1.01	0.05
B. Barley									
Sv 59132 Fertilizer level (See Table 31)									
N2, 2K2, 2P2 (perlite)	8.4	4.05	2.22	4.65	10.92	1.43	1.38	1.29	0.14
N1, 2K2, 2P2 (soil)	9.2	3.83	2.08	4.83	10.74	1.41	1.33	1.25	0.16
2N2, 2K2, 3P1 (perlite)	11.8	3.32	1.98	4.09	9.39	1.14	1.11	1.08	0.07
2N2, 2K2, 2P2 (soil)	15.3	3.35	2.01	4.28	9.64	1.14	1.12	1.08	0.06
5N2, 2K2, 2P2 (perlite)	17.4	2.59	1.62	3.37	7.58	1.04	1.04	1.01	0.03
Mean	12.4	3.43	1.98	4.24	9.65	1.23	1.20	1.14	0.09
Hiproly, Sweden	14.5	4.07	2.23	4.86	11.16	1.29	1.28	1.21	0.08
Hiproly, U.S.A	18.4	4.34	2.26	4.94	11.54	1.20	1.20	1.12	0.08
C. Oats (Whole seeds including husks)									
2221	10.2	3.71	1.93	5.94	11.58	1.57	1.37	1.28	0.29
847-4	11.2	4.17	2.15	6.38	12.70	1.50	1.38	1.28	0.22
847-139	12.9	3.86	1.99	6.02	11.87	1.46	1.33	1.25	0.21
847-23	14.5	3.83	2.12	6.37	12.32	1.47	1.33	1.25	0.23
847-90	16.8	3.75	2.05	6.43	12.33	1.41	1.31	1.19	0.22
847-236	18.4	3.92	2.15	6.47	12.54	1.38	1.24	1.12	0.26
847-235	19.4	3.94	2.36	6.95	13.25	1.44	1.27	1.21	0.23
Mean	14.8	3.88	2.10	6.37	12.37	1.46	1.32	1.23	0.23
D. Oats (Sol II)									
Seed + husk	10.74	4.10	2.40	6.54	13.04	1.40	1.33	1.24	0.16
Seed	13.35	4.06	2.15	6.67	12.88	1.33	1.27	1.22	0.11
Husk	1.51	5.75	2.26	5.49	13.50	2.31	2.03		

* Basic amino acids, lysine, histidine and arginine

of lysine, histidine and arginine with increasing protein content. There is no such correlation in oats. The DBC to KP ratio is practically unchanged in 20 and 40 mg samples in wheat and barley in contrast to oats, indicating a reaction equilibrium for wheat and barley. The difference in DBC to KP in 20 to 60 mg samples is highest for oats and fairly constant at all seed-protein levels, while this difference continuously decreases at higher seed-protein levels in wheat and barley. At all levels of sampling in wheat and barley,

the DBC to KP ratio gradually diminishes at increasing protein contents and decreasing BAA : KP, whereas a similar effect is much less marked in oats. A part of this variation could be due to differences in non-protein binding. Extremely low protein samples (e.g., oat husk, Table 10 D) give, at a constant in weight of protein, an increased DBC to KP value for this reason. At normal protein levels, such differences are relatively small, especially with the 60 mg sample (compare seed + husk to seed, Table 10

D). It therefore seems appropriate to state that a significant part of the negative slope coefficient $DBC : KP - KP$ in wheat and barley is due to the decrease of BAA with KP. In oats where $BAA : KP$ is about constant, the weak tendency of $DBC : KP$ to decrease with increasing KP could be entirely due to effects of non-protein binding. Oats thus deviates from barley and wheat in these respects.

The high-protein strain Hiproly has an amino-acid composition comparable to barley varieties of very low protein content. The DBC is about 10% higher in Hiproly than in barley lines with a similar protein content. A further scrutiny of the principles of the DBC method could be favourably carried out by selecting deviates in a breeding programme including Hiproly. Such an effort is presented in Chapter III.

A more exact method to estimate free amino groups in proteins is the method of CARPENTER (1960) adopting the fluorodinitrobenzene (FDNB) of SANGER. FDNB reacts with free epsilon-amino groups from lysine, the derivative of which is isolated after acid hydrolysis. The free epsilon-amino groups of lysine—"available lysine" (CARPENTER and BJARNASON 1970)—is severely disrupted by heating (see Chapter VI), and is correlated with the utilization of lysine in nutrition. A FDNB-copper method has been developed by SELIM (1965) for lysine screening. A lysine analysis using the derivative 2-chlor, 3-5 dinitropyridine has been employed on a large scale by VILLEGAS (1972). BOLINDER (1970) has developed an interesting microbiological method for studying amino-acid composition of hydrolysates utilizing measurements of colony size in plate assays. Screening techniques for seed-protein improvements are reviewed by KAMRA (1971) and LINDGREN (1972).

II. Inventories to estimate how cereals are selected, treated and used in Sweden

The plant breeder has direct contacts with the basic steps in the food production chain that are involved in agriculture. The demand and success of his varieties is one of his major inspirations for future planning. He also uses the tools of inquiry

on the practical level talking with farmers, seed dealers, specialized scientists, representatives from industry, and with the final consumers. In this way the plant breeder obtains a general view of how people interpret the development. It is important to obtain such relevant information to set up a programme of priorities, especially when considering the fact that product development through plant breeding is a slow process.

The problem is how to interpret the scattered, disparate information, which is often just as much the results of social economic contrasts as conflicting effects of "natural laws". Consequently it is impossible to synthesize a crop variety that will be certain to function in society 10-20 years ahead, considering only a few of the selection criteria used in a few steps in the production chain in processing cereal varieties.

With the help of the analytical criteria previously discussed, I will try to make a preliminary scrutiny of the feed-production chain in Sweden to obtain an empirical material for illustration of certain important principles.

Sweden is a sparsely populated country with a temperate climate that is often dry in the spring but, on the other hand, frequently moist at harvest time in the late summer or the autumn. Sweden is highly industrialized and the segment of her population engaged in the highly diversified mechanized agriculture is rapidly diminishing, while production volume remains rather stable. The average farm size is still comparatively small (about 25 ha.). Traditional milk and beef production is declining, whereas swine and poultry rearing is increasing (LARSSON and MUNCK 1971). Plant production is rather unbalanced (HAGBERG and MUNCK 1971), with an annual surplus of about 0.3-1.0 milj. ton mill. tons of cereals and an import of feed proteins such as soybean and fish meal (during the period 1965-67, 181,000 tons digestible protein annually).

To guarantee farmers an income comparable with that of other citizens, a high-price policy with respect to agricultural commodities has been wrought through regulations and tariffs.

The yields of the principle cereal crops were in 1970 (in millions of tons) for barley 1.9; oats, 1.7; winter wheat, 0.8; rye, 0.2; and spring wheat, 0.1. In spite of the obstacles of the temperate climate, cereal yield is comparatively high in Sweden (range: 1.5-6.0 tons/ha.; average approx. 3.2 tons/ha.) as a result of high fertilizer input,

few problems with plant diseases and a prolonged photoperiod due to the long days. The humid climatic conditions during the harvesting season (HUMMEL-GUMÆLIUS and ÅKERBERG 1967), however, result in serious grain deterioration problems.

In 1930, an annual inventory of bread-seed quality (in wheat and rye) was started in Svalöf by ÅKERMAN *et al.* This investigation of samples from representative farms offers a unique opportunity for the plant breeder to study the development of the market—how varieties are spread and how they are treated in different parts of the country. Excessive damage due to sprouting is widespread in years with humid harvest seasons (OLERED 1967). As an average during the years 1952–1962, 37% of the winter wheat, 31% of the spring wheat, and 59% of the rye samples of the Swedish production were unsuitable for baking purposes and thus degraded to feed. Only about 20% of the cereal production in Sweden is used for bread. It could be concluded (MUNCK 1964 *c*) that seed quality also in the cereal production for feed must be affected, and even more severely because bread seed is grown in favourable regions and receives priority when drying. Therefore, an inventory of barley and oats should be indispensable for establishing the plant-breeding priorities (MUNCK 1965, 1968). At any rate, it is quite clear that other quality characters than those applied to the bread-seed are of economic importance in cereals for feed.

1. Inventory of barley and oats for feed

In the years 1968–1970 an official, preliminary inventory was initiated in co-operation with The Swedish Seed Association, Svalöf, and the Swedish National Central Bureau of Statistics (HUMMEL-GUMÆLIUS, MOSSBERG and MUNCK 1969, 1971, 1972). Sampling is carried out by trained personnel at harvest and the seeds are carefully dried. Water content at harvest is determined in a small separate sample sent to Svalöf in a sealed tube. Farmers storing their own seeds are requested to send a storage sample to Svalöf in February. In the first preliminary series of investigations covering 3 years, a limited number of harvest samples (200 oats, 200 barley) and storage samples (50 + 50) were taken at places in southern and central Sweden that were representative of the best farming conditions. The samples were subjected to a great number of analyses including feeding trials with mice.

Water content in the seed is important for safe storage. The risk of fungal infection is dependent on water content, temperature and length of storage. According to WELLING (1969), barley stored at 15% and 17–18% water content should not be kept for long periods at temperatures above 20° and 10°C, respectively to retain seed viability. The harvest weather in Sweden in 1968 and 1969 was unusually favourable. The year 1970 is considered more representative of the Swedish climate. In the inventories, the water content of the barley at harvest (Fig. 3) reflects the climatic observations. In 1970, only a few percent of the samples were suitable for safe storage without drying, and there were occasional samples with very high water content (30–40%). Even in the favourable years 1968 and 1969, a majority of the samples should have been dried to maintain storage quality.

The question at hand is: How is this situation met with by the farmers? From the storage samples of 1970–71 (Table 11), we observe still very high water contents in barley and oats, 33.7% of the samples being over 18% in water content. If we extract the samples stored without drying, 30% of the samples were kept at over 20% water content and 75% stored at over 18% moisture content. An unfavourable picture was also obtained for the favourable years 1968 and 1969. The analyses of the water content reflect the farmers' behaviour with regard to drying and storing (Table 12). In the years 1968–1970, 27.2–29.2% of the samples were delivered directly to the retailers and thus escaped our checks. Storage on the farm without drying was 20.6% in 1968, 18.5% in 1969 and dropped to 12.7% in 1970, reflecting the degree of awareness among the farmers as regards the necessity of drying in this humid year. In 1970, there was also a marked change from cold- to warm-air drying. Obviously, an increased extra drying capacity was put in 1970 as compared with 1968–69. The drying in 1970 was, however, in spite of that unsatisfactory (Table 11).

Sprouting in the field is one of the obvious results of the humid climate. This reflects in a high alpha-amylase content in the seed. KIVI (1965) points out that high temperature before yellow-ripening followed by a rainy period results in sprouting. Such a situation was prevalent in central Sweden in 1969 (Table 13). Note the distinct difference in alpha-amylase activity

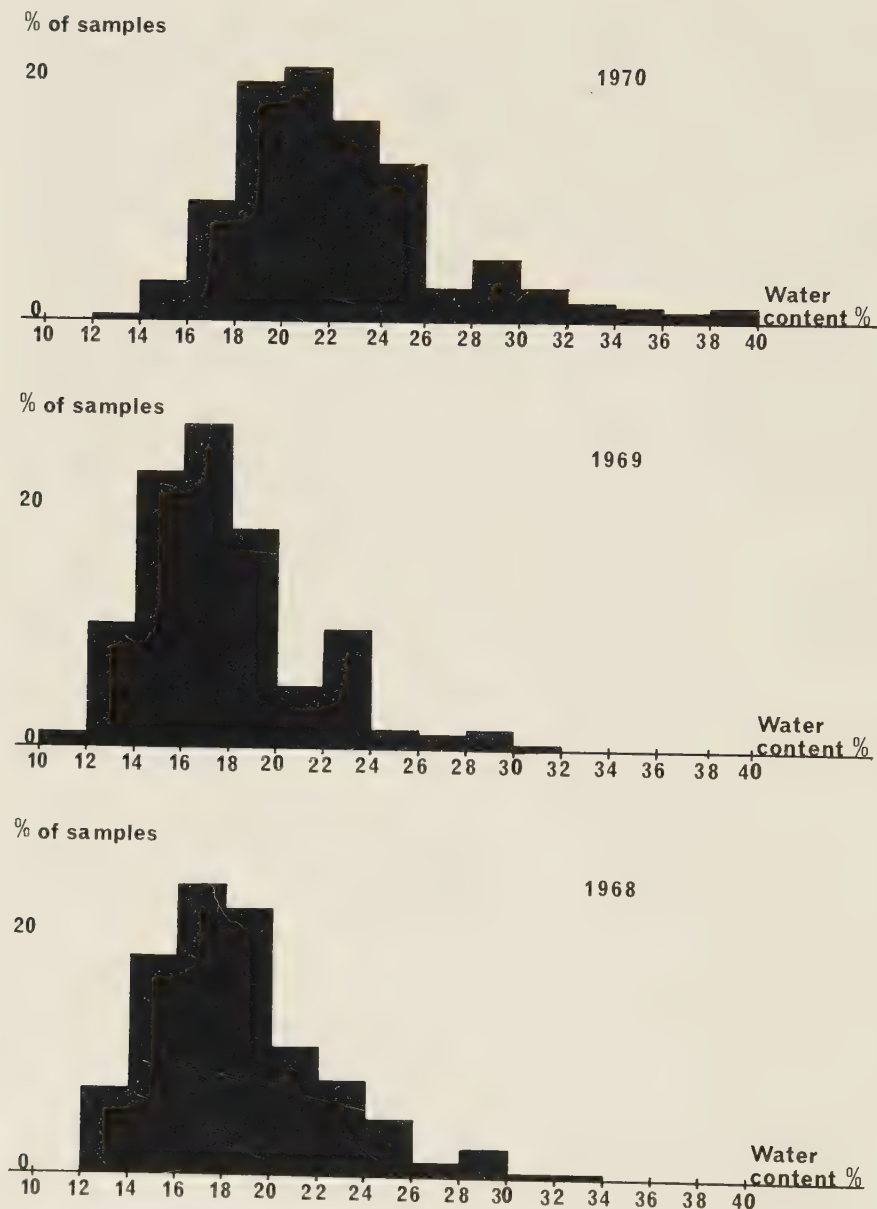


Fig. 3. Water content in barley harvest samples from the Swedish inventory of cereals for feed 1968–70.

between southern and central Sweden in 1968–70, showing the harvest difficulties in the more northern regions.

Another important reason for the inventory described is to obtain a representative material

to study screening analyses that could be used to develop production-control systems through a premium for quality. Available metabolized energy is an important economic factor in animal production. Volume weight, husk percentage of

Table 11. Water-content distribution of barley and oats samples stored at local farms in 1970–71

	% of the samples with water contents of				
	< 14.1	14.1–16.0	16.1–18.0	18.1–20.0	> 20.0
Total	9.8	29.3	27.2	25.0	8.7
Stored without drying	0.0	5.0	25.0	45.0	30.0

Table 12. Handling of barley and oats after harvest

Year	% of the growers with				
	Direct delivery without drying	Storage at the farm without drying	Cold-air drying	Warm-air drying	Ensilage
1968	27.7	20.6	23.1	28.6	0.0
1969	27.2	18.5	27.2	27.0	0.0
1970	29.2	12.7	22.5	35.3	0.3

the seed and crude fibre percentage could be used for an estimation of the available energy in oats and barley as correlated with animal experiments. THOMKE (1960 a, b) points out that husk percentage is a better estimate for unavailable energy than crude fibre in experiments with pigs and chicken. In the inventory investigations, oats are mechanically dehulled and the dehulled fraction percentage of whole seed is thus easily obtained compared with the tedious crude-fibre analyses, which cannot be used as a field method. With over-ripe seeds affected by alternate periods of dry and moist weather, however, the dehulling technique gives too high husk values.

Volume weight is influenced by several secondary factors and should be considered for crude sorting only. It can be seen from Table 14 that the estimates for available energy discussed are strongly related to each other in oats in 1968 and 1969. The lower correlation coefficients in 1970 probably reflect a more variable material as a result of climatic conditions. The dye-binding (DBC) method (Table 15) provides an outstanding estimate for crude protein according to Kjeldahl for both barley and oats ($r = 0.96^{***} - 0.99^{***}$). Because this method is very inexpensive, it could be used in practice in a payment for quality programme. In the inventory material of 1969,

there is a significant correlation in barley between g lysine/100 g protein and crude-protein percentage ($r = -0.88^{***}$), as well as between DBC/100 g protein and lysine ($r = 0.86^{***}$). There is no significant similar correlation in oats. When high-lysine barley varieties are introduced on a large scale, the DBC method will not give an accurate estimation of the crude-protein content, but still DBC will give a good reflection of the lysine content. Since lysine, at least for feeds to swine, calves and poultry, is a better quality estimate than crude protein, the DBC method in

Table 13. Median values of alpha-amylase activity in barley harvest samples from different counties in central and southern Sweden 1968–1970

County	1968	1969	1970
Stockholm	1	11	9
Uppsala	3	40	33
Södermanland	2	45	11
Örebro	4	300	11
Västmanland	6	200	22
Kristianstad	1	1	1
Malmöhus	0	1	1
Halland	3	1	1

Table 14. The correlation between dehulled seed (%) of whole seed and crude fibre (%) in oats, and between crude fibre (%) and hectolitre-weight (kg) in oats and barley

	Cereal	Year	n	Equation	r
Dehulled seed % of whole seed (x) — crude fibre % (y)	Oats	1968	44	$y = -0.25x + 27.8$	-0.95***
		1969	53	$y = -0.37x + 37.0$	-0.86***
		1970	43	$y = -0.13x + 19.7$	-0.45**
Crude fibre % (x) — hectolitre-weight kg (y)	Oats	1968	44	$y = -3.14x + 86.7$	-0.70***
		1969	53	$y = -2.09x + 77.4$	-0.72***
		1970	43	$y = -2.15x + 77.6$	-0.50***
	Barley	1968	54	$y = -2.06x + 81.1$	-0.35**
		1969	43	$y = -4.33x + 92.4$	-0.60***
		1970	47	$y = -3.51x + 88.8$	-0.44**

* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P < 0.01$

Table 15. Crude protein and its correlation with dye-binding capacity (DBC) 1968–1970

Cereal	Year	Crude protein (%)	DBC/kg—crude protein/kg r
Barley	1968	12.1	0.98***
	1969	12.9	0.99***
	1970	13.4	0.97***
Oats	1968	11.9	0.99***
	1969	13.5	0.98***
	1970	13.0	0.96***

its practical use gives more qualitative information than the Kjeldahl protein analyses.

Mouse-feeding tests were performed on storage samples that were equivalent to the respective harvest sample. Oats were fed dehulled. There was no average difference in the feeding results

due to storage, which is remarkable if we consider the high water contents. One possible explanation is that the samples had been stored at low temperatures, which kept them in good condition. Strong negative feeding effects on mouse were, however, recorded from barley samples obtained from northern Sweden in 1968 (HUMMEL-GUMÆLIUS et al. 1969). Widespread intoxication of farm animals from deteriorated cereals was observed in this region. In the inventory material of 1968–70, there is naturally a positive correlation between weight gain in mice and crude-protein content (Table 16). In barley, a positive significant correlation between mouse gain and alpha-amylase was obtained in 1969 and 1970. This reflects a better utilization of energy and protein in seeds with a high enzyme level. Alpha-amylase varied greatly in 1969 and 1970 (Table 13) independently of protein content. In oats, alpha-amylase did not affect mouse growth in 1970, although the enzyme activity was

Table 16. Correlation coefficients (r) between mouse gain and crude protein, alpha-amylase and crude fibre from harvest samples of barley and oats in 1968–1970

Component	r barley			r oats
	1968	1969	1970	1970
Crude protein	0.67***	0.68***	0.36*	0.40**
Alpha-amylase	0.22 ^o	0.41**	0.39**	0.04 ^o
Crude fibre ¹	-0.34*	-0.32*	-0.23 ^o	

¹ Partial correlation (crude protein and alpha-amylase eliminated)

highly variable in this year (Table 16). The partial correlation of crude fibre to mouse growth (excluding crude protein and alpha-amylase) in barley was significant in 1968 and 1969. Crude-fibre variation is nominal in barley. Water consumption is significantly negatively correlated to crude-protein content in barley (1969, $r = -0.74^{***}$) or positively related to starch content. Alpha-amylase content does not influence water consumption.

A joint Scandinavian barley trial (NKJ-16, MADSEN et al. 1972) was carried out with the most widely grown agricultural variety, Ingrid, at nine locations during three harvest years, 1968–1970. Mouse trials with carcass analyses (Table 17) were performed employing Swiss $\times$ CBA mice in 1968 (5), in 1969 (4), and NMRI/SPF in 1970 (2). In 1970, diets were also tested at a constant protein level of 10% (1). To compare the different years, excluding the strain effect, the same barley standard was fed in 1969 (4) and in 1970 (2), so that data from 1969 (4) considering the Swiss $\times$ CBA strain could be recalculated in NMRI/SPF terms (3). The most prominent strain difference is the low fat content of the Swiss $\times$ CBA mouse. Comparisons between years should be made with care because of the different strains used. It seems obvious, however, that the growth as measured by increase in total weight, dry matter and crude protein is retarded in 1968 (5) because of the low-protein content this year. The years 1969 (3) and 1970 (2) seem to give identical results, whereas a simulated protein decrease (1) due to starch addition to obtain 10% protein in the diet results in decreased gain.

The analysis (Table 18) considering the variance between stations demonstrates significant v^2 values for protein, lysine, and fibre percentages in the diet. This variation in chemical composition reflects significant effects on the three gain parameters and on food and protein consumption. In contrast to the effects due to station, the influence of alpha-amylase is strongly bound to harvest time (Table 18), whereas protein percentages of lysine and fibre are not significantly affected. It is interesting to note that both gain in dry substance (g) and gain in protein (g) are significantly influenced by the harvest time, which confirms the previously cited results from the inventories regarding a positive effect of alpha-amylase on gain.

Table 17. Barley trial NKJ 16: mouse feeding test on barley variety Ingrid grown 1968–70
Nine locations in Scandinavia and three harvest times, $n = 27$

Year	Mouse breed	Growth period (days)	Dietary crude protein dry matter (%)	Gain per animal		Consumed per animal						
				Total weight (g)	Dry matter (g)	Fat (g)	Crude protein (g)	Dry matter (g)	Crude protein (g)	Water (g)	PER	NR
1. 1970b*	NMRI/Spf	11	10.0	9.3	3.06	1.13	1.41	44.2	4.43	93	2.13	32.0
2. 1970a	NMRI/Spf	11	11.9	10.0	3.21	1.12	1.56	43.6	5.26	88	1.92	29.8
3. 1969**	NMRI/Spf	11	12.4	10.0	3.25	1.15	1.61	43.7	5.31	—	1.88	29.9
4. 1969	Swiss × CBA	14	12.4	10.8	3.07	0.55	1.94	48.5	5.97	—	1.71	32.5
5. 1968	Swiss × CBA	14	10.7	8.8	2.68	0.58	1.65	49.8	5.28	—	1.67	31.2

* 1970a protein standardized to 10% with wheat starch

** Data recalculated from original data (4) based on a standard barley diet, which also had been fed in 1970a

Table 18. Variance ratios (v^2) in different characters from the Scandinavian barley trial from 1968, 1969 and 1970 (see Table 17)

Source of variance	Between harvest times	Between stations
Protein (%)	0.32	5.64***
alpha-amylase (g)	6.14***	0.46
Lysine (%)	0.62	3.31**
Fiber (%)	1.67	3.12**
Gain (g)	1.80	2.73*
Gain dry substance (g)	3.82*	3.51*
Gain fat (g)	1.41	2.22*
Gain protein (g)	4.03*	3.33**
Consumed dry substance (g)	0.66	2.18*
Consumed protein (g)	0.56	3.44**
PER	0.16	1.66
NR	1.54	1.30

Table 19. The effect of barley harvest times on mouse performance in the Scandinavian barley trial (See Table 18)

The mean of the year = 100, $n = 27$

		Harvest time		
	Year	1	2	3
Consumed dry substance	1970b	100	100	100
	1970a	99	101	101
	1969	100	99	101
	1968	101	99	101
Gain in protein	1970b	97	99	104
	1970a	96	101	103
	1969	99	98	102
	1968	98	99	102
Gain in fat	1970b	98	97	105
	1970a	95	102	103
	1969	97	97	105
	1968	100	98	102
NR	1970b	98	99	103
	1970a	98	97	105
	1969	100	100	100
	1968	98	102	101

Considering the effect of harvest time on mouse performance (Table 19), intake of dry substance was found to be unaffected, whereas there is a tendency for the gain in protein, gain in fat and nitrogen retention to increase at late harvest especially in the alpha-amylase-rich year 1970.

Feeding trials by THOMKE (1972 c, d) with chicken confirm the positive effect of the late harvest of barley; on the other hand, swine and chicken performance does not seem to be affected due to harvest in the trials of MADSEN et al. (1972). The enzyme effect in barley on feeding performance is discussed in Chapter V and effects from deterioration due to storing are treated in Chapter VI.

2. Protein supplement inventory

Feed industry is a link between producers of raw materials—e.g. barley, oats, fish meal, meat meal, soybean, minerals, vitamins, and antibiotics—and the animal-feeding farmers. To have motivated existence, the feed industry should prove a superior skill in combining different nutrients, such as carbohydrates, proteins, fats, etc., in such proportions that a feed composite is obtained that insures optimal animal productivity in relation to the economic input. From the previous discussion, we can conclude that cereals for feed have a highly variable composition as regards important amino acids, available energy, and specific dietary qualities. The feed producers must combine this material with more expensive supplements mainly of proteinaceous nature in order to produce feeds that meet the nutritional needs of the different farm animals. Within raw materials, the crude protein analysis is likely to give some representation of the amount of essential amino acids like lysine and methionine. However, heat treatment during processing may make these amino acids unavailable to the animals. The epsilon amino groups of lysine may react as is discussed in Chapter VI, and from the point of view of nutritive value such an altered lysine residue is likely to be lost. The Kjeldahl analysis and, to some extent, the lysine analysis according to MOORE and STEIN will not always reflect these changes in lysine availability.

To illustrate this problem, a small inventory of commercial protein supplements (MOSSBERG and MUNCK 1971) was made (Fig. 4) considering 20 fish meals (black dots), 15 soybean meals (crosses) and 8 meat meals (circles). In Fig. 4, lysine g/16 g N, according to MOORE and STEIN, is related to crude-protein percentage of dry

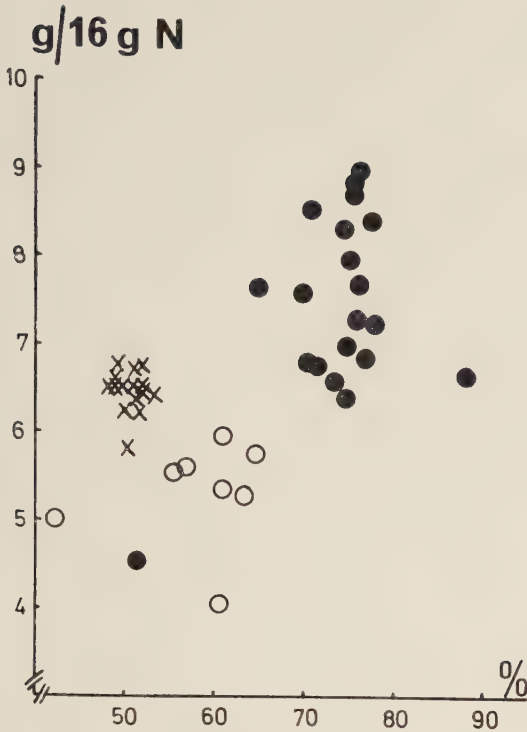


Fig. 4. Lysine content in crude protein (y-axis) and crude-protein percentage (x-axis) in fishmeal (dots), meatmeal (circles) and soy-beanmeal (crosses) from a preliminary feed supplement inventory in 1969.

substance. A great variation with respect to lysine content is observed for fish meal and meat meal, while soybean meal is much more uniform in lysine. Fish-meal samples holding between 70 to 80% crude protein of dry substance are sold as 68% (calculated on the undried material) guaranteed protein meals. Lysine in these meals varies with 50% from about 6 to 9 g/16 g N. As lysine is much more representative for the value of use of fish meal in swine and chicken diets, lysine analysis should be preferred before Kjeldahl as a guide in feed manufacturing provided a suitable screening analysis could be developed.

A still more representative estimate of nutritive value is the measurement of the free epsilon amino groups of lysine, that is available lysine according to CARPENTER (1960). In Fig. 5, it is shown that available lysine (g/16 g N) is from practical point of view sufficiently well correlated

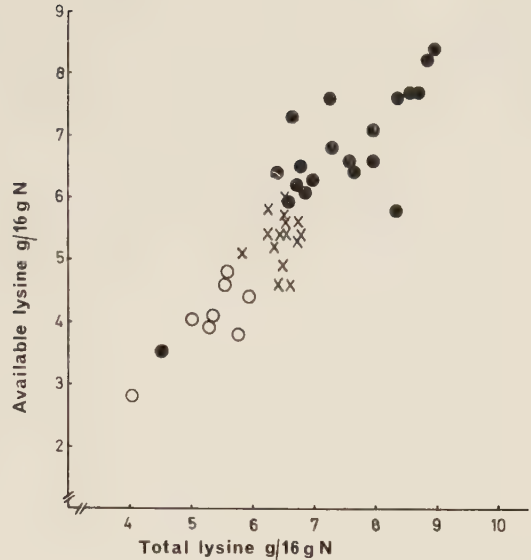


Fig. 5. Relation between available lysine (g/16 g N) and total lysine (g/16 g N) in feed supplements presented in Fig. 4.

with the conventional MOORE and STEIN lysine analysis (g/16 g N) in comparison with the crude-protein percentage—lysine g/16 g N relationship in Fig. 4.

It is obvious that a rapid analysis for lysine or available lysine would be a very useful tool in the economic production of feeds. The dye-binding (DBC) method could at least partially solve this problem. By using oxalic-acid additives in the dye solution (UDY, 1971; MOSSBERG pers. comm.), the interfering calcium ion is eliminated. The DBC method could thus be used as an available-lysine estimate for fish meal, meat meal and composite feeds in addition to its applications in cereals described in Chapter I. A few feed factories in Sweden and Denmark are already using the DBC method daily in their production control.

3. Inventory of feed composites

To study the present situation regarding the variation in important nutrients in slaughter-swine feeds, a preliminary inventory (Tables 20 a, 20 b, 21 and 22) was performed comparing three feed factories (I, II and III)

Table 20a. Protein and mineral composition of commercial, slaughter pig feeds

Values in italics denote range of variation

	Feed factory			
	I	II	III	III*
Samples n	15	21	13	
Water content (%)	12.0 ± 0.3 <i>11.6 - 12.7</i>	11.6 ± 0.5 <i>10.7 - 13.2</i>	12.1 ± 0.7 <i>10.6 - 12.6</i>	< 12.0
Crude protein (%)	16.7 ± 0.5 <i>16.0 - 17.7</i>	16.2 ± 0.8 <i>15.1 - 17.5</i>	16.5 ± 0.5 <i>15.6 - 17.3</i>	> 16.0
Lys**	5.13 ± 0.11 <i>5.0 ± 5.4</i>	4.95 ± 0.22 <i>4.7 ± 5.6</i>	5.17 ± 0.15 <i>5.0 ± 5.4</i>	
Met**	2.09 ± 0.05	1.93 ± 0.07	1.88 ± 0.06	
Cys**	1.89 ± 0.07	1.94 ± 0.07	1.86 ± 0.08	
Arg**	5.72 ± 0.13	5.63 ± 0.13	6.29 ± 0.11	
Pro**	7.01 ± 0.44	7.71 ± 0.22	6.61 ± 0.22	
Glu**	19.18 ± 0.36	19.07 ± 0.29	18.21 ± 0.35	
Undigest.*** protein (%)	1.1 ± 0.1 <i>1.0 - 1.3</i>	1.2 ± 0.4 <i>1.0 - 2.6</i>	2.5 ± 0.4 <i>1.1 - 2.9</i>	
P (%)	0.61 ± 0.09 <i>0.58 - 0.68</i>	0.56 ± 0.06 <i>0.49 - 0.74</i>	0.76 ± 0.05 <i>0.67 - 0.84</i>	> 0.7
Ca (%)	0.78 ± 0.09 <i>0.68 - 0.90</i>	0.81 ± 0.11 <i>0.64 - 1.00</i>	1.17 ± 0.08 <i>0.98 - 1.28</i>	> 0.9
Fe 10 ⁻⁴ (%)	104 ± 76 <i>63 - 161</i>	96 ± 19 <i>51 - 128</i>	127 ± 27 <i>94 - 158</i>	

* Composition according to guarantee from producer III

** Amino acids (g/16 g N), Met and Cys analysed by the oxidation procedure

*** Undigestible (pepsin) protein (N × 6.25) % dry weight

from the province of Scania (Skåne) between December 1969 and April 1970 (MUNCK 1971). The factories regularly delivered feed in bulk (crushed pellets) to three large swine stables on the same farm (Table 21). The stables were of similar construction, although environmental-influenced differences could not be completely disregarded. Samples were taken from each delivery and were extensively analysed (Table 20 a, b) as well as fed to mice (Table 21).

The swine were checked by a veterinarian and were examined with regard to slaughter quality. After the feeding period, the feed factories were asked to supply their feed formulas. Because of the confidential nature of the formulas, the relative composition (Factory I = 100) is given in Table 20 b.

The chemical composition of the diets reflects the different raw materials used. Some analyses could thus be used as markers to prove the presence of certain components. Thus, feed factory III (Table 20 b), which had included broad bean in the diet, is the only one having haemagglutinins which is characteristic for the broad bean (MUNCK et al. 1972 b). Tryptic inhibitors occur in large amounts in raw soybean meal, which

therefore is heat-processed to decrease their activity. All three sources of feed are supplemented with soybean meal. Factories with the highest maximal value of tryptic inhibitors are thus including the greatest amounts of soybean meal in their feeds.

The alpha-amylase activity (Table 20 b) is recognized to trace the harvest conditions of the cereals used in the mixes. This enzyme is comparatively stable at temperatures reached during milling and pelleting. Other sources than cereals, such as fish meal, meat meal and beans are devoid of or contain only a small amount of alpha-amylase activity. It is concluded that factory II occasionally has high levels of alpha-amylase in its feed, which is characteristic of cereals produced in central and northern Sweden in 1969 (Table 13).

The Ca : P ratio should be fixed at about 1.3 (NIELSEN et al. 1971) to obtain high feed efficiency and good bone performance for swine. Calcium is added in the form of lime or bone

Table 20b. Protein and mineral composition of commercial, slaughter pig feeds

	Feed factory			
	I	II	III	III*
Crude fat (%)	5.6 ± 0.3 4.9 – 6.0	4.9 ± 0.5 3.5 – 5.8	6.1 ± 0.3 5.7 – 6.7	> 5.5
Crude fibre (%)	4.7 ± 0.6 3.8 – 5.7	4.7 ± 0.9 3.8 – 5.8	5.2 ± 0.9 3.9 – 6.4	< 4.5
Alpha-amylase (units)	6 1 – 50	38 1 – 460	5 2 – 12	
Falling number (min)	140 69 – 320	157 62 – 328	73 65 – 105	
Haemagglutinins (units)	0.0	0.0	1.0 0 – 2.5	
Tryptic inhibitors (units)	0.23 0 – 1.24	0.28 0 – 1.01	0.20 0 – 0.60	
<i>Relative raw material composition I = 100</i>				
Barley	100	144	109	
Oats	100	59	127	
Wheat	100	50	80	
Fish meal	100	94	104	
Meat meal	—	+	—	
Soybean meal	100	78	39	
Broadbean	—	—	+	
Fat	100	67	93	
Minerals	100	103	144	

* Composition according to guarantee from producer III

meal. Most feed sources except meat-bone meal and occasionally fish meal are devoid of calcium. Phosphorus is, on the other hand, more uniformly spread among the raw materials. Thus, calcium could be employed as an indicator of the efficiency of feed mixing. In Table 20 a it is seen that calcium varies much more than phosphorus, reflecting the difficulties in getting the feed mixed accurately, a critical problem within the feed industry (see discussion by SUNDSTØL 1970). This problem is also illustrated in Table 20 a by the great fluctuation in iron content. It is observed that factory III has a high level of minerals as compared with factories I and III. This seems to be partly due to the inclusion of more minerals (Table 20 b). It is also likely, however, that factory III uses a low-quality fish meal rich in minerals and low in available protein judging from the high content of undigestible protein found in these feeds (Table 20 a). Feed factory II has most cereals and feed factory III uses most beans (Table

20 b). This affects amino-acid composition (Table 20 a) so that glutamic acid and proline are especially high in feed II, reflecting the prolamines of barley, whereas arginine is elevated in feed III mostly due to the bean increment, which is rich in this amino acid.

With regard to swine performance (Table 21), feed I gives a growth period that is one week shorter than those of feeds II and III. Feed efficiency is lower with feed III (3.75) as compared with feeds I (3.25) and II (3.29). The same tendency is also observed in the mouse trials—the differences, however, not being statistically significant. The unfavourable feed conversion for feed III is not due to less intake of digestible crude protein, lysine or sulphur amino acids. Carcass quality is lowest in feed I (Table 21). As this feed results in excellent growth and feed conversion, it is likely that it has been fed too richly. There were no differences in carcass fat percentage in mice between the different brands of feed (unpubl.).

Table 21. Nutritional quality of commercial, slaughter pig feeds fed to pigs and mice

	Feed factory		
	I	II	III
<i>Swine</i>			
No. of animals	1029	1811	1607
Weight gain (kg)	67.9	69.8	65.5
Feeding period (days)	122	129	129
Feed consumption (kg)	221	230	245
Feed efficiency (kg/kg)	3.25	3.29	3.75
Mortality (%)	2.3	2.6	2.0
<i>Consumed</i>			
Crude protein (kg)	36.9	37.2	40.5
Digestible crude protein (kg)	34.5	34.5	34.4
Lysine (g)	177	184	209
Met + cys (g)	147	144	151
Extra premium carcass quality % of delivered animals	57	72	75
<i>Mouse*</i>			
No. of animals	60	84	52
No. of diets	15	21	13
Weight gain (g)	11.5 ± 0.5 10.6 – 12.4	11.2 ± 0.5 10.4 – 11.9	11.3 ± 0.6 10.3 – 12.3
Feeding period (days)	14	14	14
Feed consumption (g)	47.3 ± 1.0 46.0 – 49.1	47.4 ± 18 43.8 – 50.2	49.7 ± 1.8 48.6 – 53.0
Feed efficiency (g/g)	4.15 ± 0.15 3.9 – 4.3	4.24 ± 0.09 4.1 – 4.4	4.42 ± 0.22 4.1 – 4.7

* Swiss × CBA 1 × 4 per diet, 11 day-experiment

4. Discussion

With the introduction of the combine, proper harvesting and storage of cereals under moist climatic conditions was rendered more difficult as compared with the automatic-binder technique, which included weathering and storage on straw and threshing during the winter. The latter system was characterized by a great expenditure of labour, but permitted safe handling and storage at high water contents, especially during the warmest period of autumn. The use of the combine delayed the harvest and exposed the crop to the autumn rains. The threshed grain had to be dried to a much lower water content to be stable for storage compared to unthreshed grain. The combine, which was developed for the dry harvest climate of the American prairie, was thus unsuited for Scandinavian conditions especially considering the northern parts. The problems in

swine feeding due to deteriorated grains were accentuated, especially in Denmark during the 1960's. A research committee to study feed-grain quality was established in Denmark in 1963 and delivered a report in 1970 (PEDERSEN 1970) with emphasis on deterioration owing to fungous infections. The reason that these problems are given more attention in Denmark than in other parts of Scandinavia seem to be due to a mild winter climate, which influences the limit of the water content for safe storage, as well as an unsatisfactory legislation in the cereal market. As a reminiscence from the old binder era, Denmark still holds to a 17% water content as the basis for price setting. The other Scandinavian countries have lowered this limit, Sweden to 15% with dry-substance corrections down to 13%.

According to observations by the present writer there seems to be a negative attitude among the farmers towards complete feed rations

Table 22. Simulation of maximal and minimal levels of lysine in a slaughter pig-feed composite

Raw material	Composition %	Lysine (%) in raw material			Lysine (%) in feed composite		
		Table value	Inventory		Table value	Inventory	
			min.	max.		min.	max.
Barley	81.5	0.40	0.36	0.45	0.83	0.79	0.87
Fish meal	5.3	5.0	2.0	6.9	0.83	0.67	0.93
Soybean meal	8.3	2.9	2.3	2.9	0.83	0.78	0.83
Fat	3.0						
Premix	1.9						
	100.0						
Maximal range						0.60	0.97
Full-feed inventory: I						0.82	0.92
II						0.74	0.93
III						0.80	0.90

for swine in Denmark, which could be tentatively explained by the inferior reliability of commercial rations. Feed blending on the farm with home-grown cereals and feed concentrates is thus favoured.

The data from the Swedish barley and oats inventory regarding water content confirm that farmers take great risks with regard to conventional seed quality (seed viability) in the storage of their grain. The moderate storage damage observed can be explained by the widespread storage of grain on the floor in thin layers that, in combination with cold weather, precludes increased temperatures caused by microbiological processes. If storage samples were taken and analysed in May-June, a greater number of fungous-infected samples should be expected, which is known to be the case from practice. The interpretation is also made more difficult owing to favourable enzymatic effects at high water contents that improve the nutritional value of barley, e.g., for chickens and mice.

In Swedish feed industry, limited resources are allocated to analysis and utilization of the variation of nutrients in order to produce inexpensive and safe feeds (LARSSON and MUNCK 1971; MOSSBERG and MUNCK 1971). Protein supplements are often bought on contract on the basis of the crude-protein content. Cereal shipments are seldom differentiated with regard to quality in storage. They are more or less commingled in the large silos. Tables of approximate

average values of—e.g. energy, lysine, crude protein, etc.—are used in calculating the feed formulas. To reach a high probability of fulfilling certain nutritional standards, a large number of commodities are often used to minimize the risks for one ingredient to influence negatively the complete-feed composite. The feeds are often nutritionally optimized with regard to indirect (apparent) criteria, like crude-protein content and rarely with regard to e.g., lysine or available-lysine content. The knowledge of the variation of important nutrients gained through the inventories could be used in a statistical computation to simulate the optimal production quality and economy in the manufacture of complete feeds. Here, however, only the extreme effects of lysine variation will be discussed (Table 22). Barley, fish meal and soybean meal are mixed to obtain an optimal lysine content of 0.83%, according to current table values. The inventories previously referred to reveal a variation in lysine from 0.36 to 0.45% in barley, from 2.0 to 6.9% in fish meal and from 2.3 to 2.9% in soybean meal. In Table 22, showing the partial effects of variation of barley, fish meal, and soybean meal on the complete feed, it is seen that especially fish meal exerts a great influence on the lysine content of the complete feed.

If the raw material samples highest in lysine were combined, lysine in the complete feed would be 0.97% compared with 0.60% for the lowest

possible combination. If we compare these figures with the ranges found in the complete-feed inventory (Table 20), the minimum lysine level is very near the recommended 0.83%, whereas maximal levels are 0.90–0.93%, indicating an extra input of lysine-rich protein for safety. An improved production control, e.g. with the DBC method, could spare this costly extra protein that often, according to veterinary practice, has negative effects on the health status of the swine, e.g., as reflected by an increased incidence of dysentery.

Feed number III in the feed inventory is 8% inferior in economic yield as compared with feed number I (MUNCK 1971). This difference is equivalent to the margin of existence for the swine producer. It is difficult from the analyses to directly point out the reason for this inferiority. The high content of undigestible protein in feed III may be due to an overheated, low-quality fish meal with impaired availability of lysine and sulphur amino acids. The mineral feed balance seems to be too high in calcium, and the use of broad bean, which is very difficult to harvest and dry under southern Swedish conditions, could also signify additional risks. An increased experience through similar inventories could greatly enlarge the understanding of how complete-feed quality depends on the previous steps in the production chain and permits more definitive conclusions. From the complete-feed inventory (Table 21), it can also be concluded that it is of limited use to produce a very nutritious growth-promoting diet, exemplified by feed I, if the swine rearer is not instructed how to feed it. The effect in this case was a more rapid growth but inferior carcass quality, which probably would have been corrected by a less intensive feeding, retaining the favourable feed conversion at a higher percentage of premium carcass classification.

The present situation of the Swedish slaughter swine producer reflects the most varied implications engendered by the other steps in the production chain (MUNCK 1971). He buys piglets, feeds, stables, utilizes veterinary checks, and engages labour. These resources, then, are integrated to produce a high, lean-meat carcass that could be sold to the slaughterery at a premium price and assure him a reasonable profit. The swine producer has very limited possibilities today to supervise and control the optimization of

the different production factors. By integrating the different production steps in his business, including cereal and piglet production as well as the feed mixing, he can exert an empirical control over the production-management network, if he can afford the big investments that are necessary. Thus, for instance by drying cereals properly, deterioration owing to fungous infection (see Chapter VI) does not need to be sampled and studied further.

In the production chain the consumer demands for high-quality lean bacon have been successfully communicated by the slaughtereries to the swine feeders by premium payments for high carcass quality. A comparison between the baking and feed industry suggests that the Swedish feed industry has been less successful in promoting the quality concept (LARSSON and MUNCK 1971). This lack of parity, then, is more or less tantamount to the animal breeders not having been efficiently organized to define all the economic requirements for swine feeding and then impose them on the feed producers. The latter stage in the production chain has consequently not exerted the pressure needed on the producers of the raw materials for an optimization of their contribution to the production chain. It is obvious that each step selects its means of production to be transformed into saleable products that will give the greatest profit. Moreover, each step has to recognize the accomplishments of the previous steps in its own production to optimize the economic performance of the entire production chain.

From the above discussion, we can conclude that if the feed industry wants to expand into the complete feed market it will have to make an organizational contribution leading to safer feeds, also with respect to the cereal constituents. This implies more effective control, as well as adequate bonuses to stimulate rapid drying and to prevent deterioration and subsequent losses in swine production. So far, there has been no breakthrough in the negotiations between the farmers' associations, feed factories and state officials as to the proper division of the costs for such a programme. On the other hand, there has been no general approach to calculate the losses in the animal-husbandry sector because of nescience as regards cereal quality. The cereal inventory presented could serve as a starting point for studying the *methodology* for such an

investigation, which in its full development could give a reasonably adequate and objective description of the gains and losses in the production chain in relation to the economic returns involved. These studies may be followed up with veterinary checks and physiological studies of the risks involved in feeding deteriorated cereals. Such an inventory may serve as a necessary background for meaningful negotiations regarding legislation and control to promote an overall optimization of the production chain. From such a material, adequate control methods could also be developed employing direct or indirect analyses for, e.g., energy, protein content, lysine, and degree of deterioration.

5. The role of the inventories for establishing priorities in development programmes

The 40 years of bread-seed inventories at Svalöf have, to a great degree, influenced the wheat-breeding programme, but particularly that of rye. Priorities have been drawn up for selection of sprouting-resistant varieties that can endure a rainy harvest season. In the beginning of the 1960's, the greater part of the rye production was unsuitable for crispbread and related bakery products. The industry is now able to purchase its own needs of rye in Sweden as a result of the release of the low alpha-amylase variety Othello (HAGBERG and PERSSON 1971), and also as a result of the implementation of an appropriate control system through contractors and through premium payments for quality estimated by the falling-number method.

The situation for the cereals destined for feed is much more complex with regard to the nutritional effects caused by deterioration. It is obvious that the risks for mycotoxicosis and losses in nutrients have both to be weighed against the favourable effects, which could be of economic importance, e.g., for poultry. The risks of fungous infection will be discussed in Chapter VI with regard to Scandinavian conditions. Even if severe cases of mycotoxicosis in Scandinavia seem to be rather rare, even a low incidence must be considered. The Swedish slaughter-swine

producer saves approximately \$2 per animal that is sold for about \$60. Even a very low incidence of mortality owing to mycotoxicosis in a production unit, such as 1 per 1000 feed lots, may thus be of great importance for the economy of the individual producer.

From a Swedish plant breeder's viewpoint, it is reasonable to consider whether a 1-week earlier maturation of a barley variety that results in a safer harvest could have as much influence on the nutritional quality, as, e.g., a 2–3 % absolute increase in seed protein. On the other hand, plant breeders could also tentatively promote enzyme-rich barley varieties for poultry that are suitable for a controlled form of favourable treatment, viz. through a deliberately late harvest followed by efficient drying. It is also obvious that new economic harvesting, drying, and storage techniques could decisively influence the priorities in plant breeding.

With regard to the content of important nutrients in cereals for feed, higher energy and protein content, as well as a more balanced amino-acid composition are profitable, although these parameters preferably should be expressed on a per hectare rather than on a per dry-matter basis. Thus, the following tentative programme may be proposed for Swedish cereals for feed:

- (1) Without losses in seed yield, introduce varieties that are:
 - a) Resistant to weather damage through characters, such as early maturity, straw strength and low alpha-amylase.
 - b) High in available energy, which implies low-fibre content in oats and barley and/or high-enzyme content in barley.
 - c) Rich in essential, available amino acids, such as lysine, sulphur amino acids, and threonine, at a high level of crude protein.
- (2) The development of new harvesting, drying, and storage methods and techniques allowing cereals to be economically and safely handled even at very high water contents (50%) without losses in feeding performance.
- (3) The development of fast, direct or indirect, screening analyses for economically desirable quality characters which could be used in commerce in a payment for quality programme as well as in plant breeding.

III. Inventories of the genetic variation in cereals for characters of priority and the analysis of the deviates to facilitate plant breeding

Selection for characters in plant breeding that are of direct economic importance for the grain farmer, such as disease resistance, straw strength, seed quality, and yield, is much easier to justify in a plant-breeding programme than quality parameters, such as lysine content, which exert their importance in the later stages of a production chain. When breeding for quality, the plant breeders must receive support from specialists who can reduce the complicated concept of, for instance, the nutritional value of cereal protein to a few simple and inexpensive screening analyses giving pertinent information of economically limiting factors.

As early as 1895, selection was started for high and low contents of crude protein and oil at the University of Illinois at Urbana in the U.S.A. (DUDLEY and LAMBERT 1969). After several decades of continuous selection, protein content ranged from 5.2 to 25.2%. The high-protein accumulation was, however, mostly due to the zein fraction, which was low in lysine (ALEXANDER et al. 1969). Yield capacity and seed size were depressed in the high-protein selections. NELSON (1969b) pointed out that high crude-protein percentage of seed meal is not a relevant parameter in plant breeding because such selections also tend to favour lines high in protein owing to poor starch synthesis. Breeding for protein content should thus be performed with a concomitant consideration of maintaining seed size. The findings of the high-lysine maize mutant opaque-2 (MERTZ et al. 1964) and floury-2 (NELSON et al. 1965) opened up new perspectives for breeding for a radically altered amino-acid balance in maize and other cereals. This discovery was made when analysing a limited number of morphological maize mutations with complete amino-acid analyses according to MOORE and STEIN. The opaque-2 and floury-2 milky phenotypes could not be distinguished from each other. Similar mutations like floury-1 and opaque-5 were not associated with an altered amino-acid pattern (NELSON 1969 b). The endosperm traits could be used as markers for simple selection for high-lysine

segregants in cross-breeding programs. Opaque-2 is recessive and floury-2 is semidominant (NELSON 1969 b). Inspired through these discoveries, inventories of protein quantity and quality were initiated in wheat (JOHNSON and MATTERN 1969), in sorghum (PICKET 1972), in rice (JULIANO et al. 1968) and in barley (HAGBERG and KARLSSON 1969; MUNCK et al. 1969). A positive variation in protein content was found in all cereals, and it was generally concluded that it ought to be possible to develop high-protein lines without sacrificing yield. In rice and barley an increase in protein content should be particularly advantageous because of their better amino-acid balance from a nutritional standpoint as compared with wheat, maize and sorghum. Also differences in amino-acid composition were recorded in the inventories cited, lysine being generally negatively correlated with the crude-protein level. High-lysine deviates with respect to this relationship were found in wheat, sorghum and barley, but confirmed to be heritable only for the barley selection CI 3462-Hiproly (MUNCK et al. 1970). Later seven new high-lysine mutants in maize were found all displaying additional morphological changes (MISRA et al. 1972).

Another approach is to increase variability through mutations. SCHOLTZ (1960) has carried out extensive screenings in mutation material of barley to discriminate mutants for high crude-protein content. An increase of 20% was possible without influencing the yield or the amino-acid composition. DOLL (1971, 1972 pers. comm.) has utilized the DBC-Kjeldahl technique in mutation breeding with barley. Six mutants were obtained from the screening of 10,000 lines. One of these mutants, recently found, appears to have a lysine content in crude protein above Hiproly (INGVERSEN et al. 1972).

1. Preparing plant breeding for high-lysine content in barley

A. Experiments

In the screening work at Svalöf, about 2,500 entries from the World Barley Collection (USDA, Beltsville, Maryland, U.S.A.) grown in Sweden were screened for dye-binding capacity and Kjeldahl nitrogen (KP). Because of the comparatively small response of DBC in relation to the large changes in lysine or BAA, one cannot afford

to reduce the degree of accuracy in DBC and KP. In the screening work for DBC, 0.5 g samples were taken to 20 ml dye solution according to MOSSBERG (1969). Double analyses were made. All values were plotted in DBC-Kjeldahl protein diagrams. For the theoretical work (Fig. 6–11, Tables 23–28), a constant weight of 60 mg protein ($N \times 6.25$) to 20 ml dye solution was used according to MUNCK et al. (1971). It is important to check deviates selected from the DBC:KP analyses by growing them in different years and in other localities together with normal varieties with similar KP content. Due consideration has to be given to the negative correlation between lysine g/16 g N and KP when judging the changes in lysine.

In the first thousand lines from the World Barley Collection, a high-protein line (CI 3947 = Hiproly) was found to have an increased DBC (Fig. 2) that deviated from the DBC–KP regression (HAGBERG and KARLSSON 1969; MUNCK et al. 1969), and an improved level of lysine of 20–30% as compared with normal lines with comparable protein content (Table 10, B). Hiproly that was grown in Sweden, in the United States and in the Canary Islands was confirmed also to be high in lysine. The discovery of the lysine deviate Hiproly gave impetus to investigations to determine how certain apparent factors, like DBC, KP, lysine and other amino acids, kernel morphology, plant habit, day-length response, and yield, are related to one another and transmitted to subsequent generations after crossing with high-yielding varieties. Such a study trying to assess the phenotypical effects of a proposed genetic complex is needed to understand how and if the deviate can be used in a plant-breeding programme promoting quality without depressing yield. Thus, the strategy is to apply screening techniques in these investigations that involve important parameters in pedigree analysis and to collate deviates in the various classes with regard to amino-acid composition. In this way also the efficiency of the DBC method could be studied.

Hiproly (CI 3947) was found to be very low-yielding for Swedish conditions (giving about 30% of high-yielding varieties). It was introduced into the World Barley Collection in the 1920's from Egypt with a note on its Ethiopian origin (WIEBE pers. comm.). Two lines (CI 3947, CI 4362) were propagated from the original collection having the same habit (two-row, naked, short *erectoides*). Both lines were high in protein, but remarkably enough, CI 4362 was found to be low in lysine (Table 27), as first reported by WIEBE (pers. comm.). Hiproly (CI 3947) has shrivelled seeds and a lower, 1000-kernel weight and yield capacity than its sister line (CI 4362).

It could be concluded that CI 4362 was more or less isogenic to Hiproly. The question was now if the amino-acid composition and content of Hiproly could be introduced into high-yielding lines.

Crosses were made to four high-yielding barley lines (HAGBERG et al. 1970; MUNCK et al. 1970) with Hiproly as the maternal parent. The reciprocal cross gave a low seed set under greenhouse conditions. The yield from F_1 plants was found to be normal in DBC, whereas KP content varied greatly (MUNCK et al. 1970). The F_2 plants were also screened, and here two classes appeared with high and low DBC's over a great range of KP values. Two of the four crosses segregated in ratios that were not significantly deviating from a 3:1 ratio (low to high DBC, respectively), whereas in the other two crosses there were significantly fewer high DBC segregants. It was concluded that the high-lysine character was recessive, with indications of modifiers being present in some combinations. The gene was inherited independently of KP content. By crossing with markers in the different chromosomes and analysing the F_2 with DBC and KP, KARLSSON (1972) successfully localized the gene to chromosome seven.

F_2 plants (from all four crosses), that were high and low in DBC were verified by amino-acid analyses. In accordance with the maize mutants, several other amino acids are affected (MUNCK et al. 1970). The symbol "lys" was suggested for the high-lysine gene from Hiproly barley by KARLSSON (1972) and MUNCK (1972 b), which symbol should be revised when the basic gene action is better understood.

Because of the association of the high-lysine maize types with visible morphological changes in the endosperm, it seemed appropriate to compare seeds from Hiproly and its sister line with high-lysine and normal seeds from F_2 crosses and with normal parents. High-lysine seeds were found to have a hard flinty endosperm. In meal preparations dyed with acilane Orange and studied in the light microscope, the matrix proteins were found to adhere characteristically to the starch granules, whereas in normal seeds separate, distinct starch granules could be observed in great numbers (MUNCK et al. 1970). Meal particles from a single seed could be removed and studied employing a scratch technique without affecting the viability.

F_3 seeds from amino-acid-verified F_2 plants were studied with regard to the endosperm-meal character and sown in growth chambers at 24 and 14 hours of day length at the same amount of light (lux/day) (HAGBERG et al. 1970). The plants were harvested individually and the date of ear emergence, grains per plant, 1000-kernel weight and yield were determined. F_4 rows representing a multiplication of each F_3 plant were analysed for DBC, KP, 1000-kernel weight and morphology. In addition, the particle size of the meal was estimated in the F_4 material by sieving in a 120-mesh screen after milling in a falling-number hammer mill. DBC is affected by particle size of meal and milling conditions (MOSSBERG 1969). The hardness of the seed may interact with milling and thus the percentage of coarse meal should be known when interpreting the DBC analyses.

In Fig. 6–11, DBC, KP, BAA, and lysine g/16 g N are described for normal and high-lysine

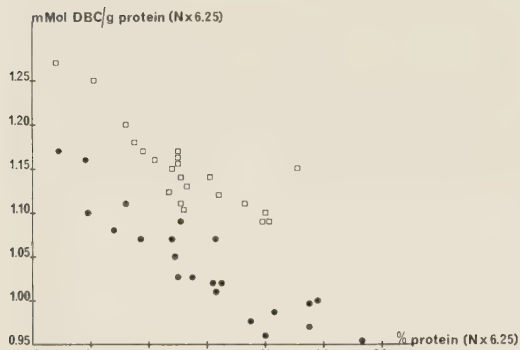


Fig. 6. Segregation for dye-binding (mMol/g protein) at different crude-protein levels in F_2 plants from Hiproly $\times$ normal-lysine barley. High DBC plants are indicated with squares and normal DBC plants with dots.

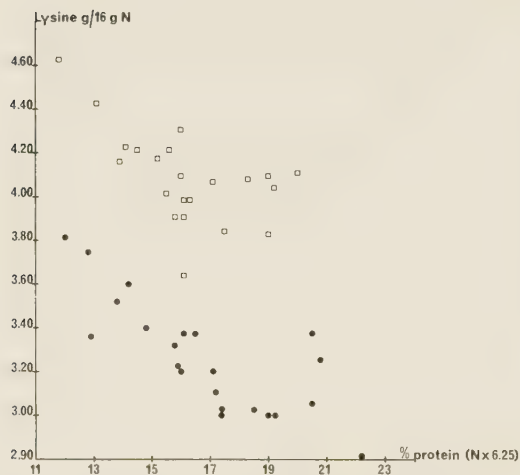


Fig. 7. Segregation for lysine (g/16 g N) at different crude-protein levels in F_2 from the material in Fig. 6.

plants segregating in the F_2 that were selected with respect to their amino-acid contents. This material constitutes the basis for the lines selected in the F_3 and studied in the F_3 and F_4 generations (Table 23). It is obvious from Fig. 6 that normal plants (circles) and high-lysine plants (squares) constitute two separate populations with regard to the negative correlation DBC mMol/g N $\times$ 6.25 – N $\times$ 6.25 (DBC: KP – KP). This

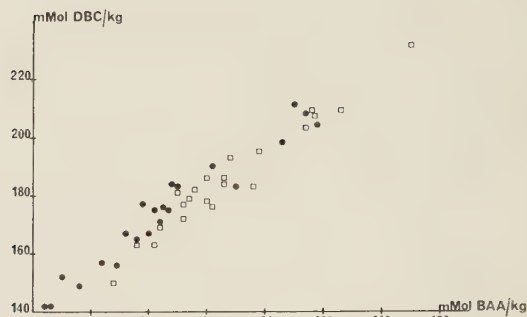


Fig. 8. Relation between dye-binding (mMol DBC/kg) and basic aminoacids (mMol/kg; lysine, histidine and arginine). Material from Fig. 6.

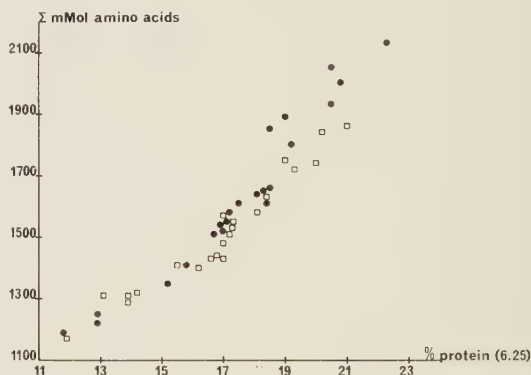


Fig. 9. Relation between molar sum (mMol) of 18 analysed aminoacids and crude protein percentage according to Kjeldahl in the material from Fig. 6.

pattern is confirmed by the lysine analyses (Fig. 7), displaying two different classes with regard to lysine g/16 g N at a wide range of KP contents. Lysine g/16 g N is negatively correlated with crude protein, but with a lower correlation coefficient for the high-lysine class than for the low-lysine one (MUNCK 1970a). As is evident from Fig. 8, mMol DBC/kg is positively correlated with mMol BAA/kg, confirming the results by MOSSBERG (1969) in a pooled material of six cereals. The KP content (Fig. 9) is also correlated with the sum of the different amino acids (mMol) obtained through a complete analysis without oxidation (tryptophane excluded). In small samples (single seeds), the sum of the amino-acids can

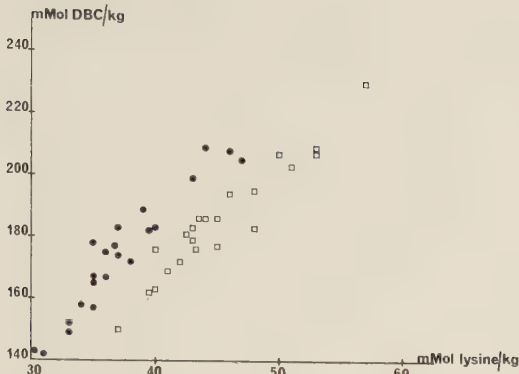


Fig. 10. Relation between dye-binding (mMol/kg) and lysine (mMol/kg) in high-lysine (squares) and normal-lysine (dots) barley segregants from Fig. 6.

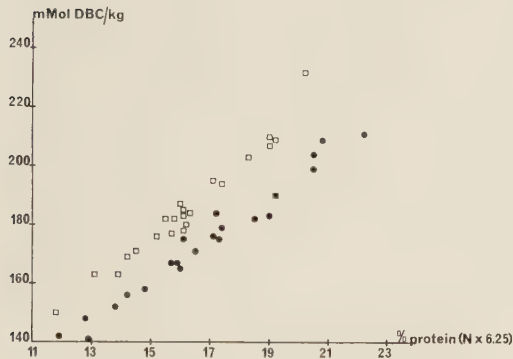


Fig. 11. Relation between dye-binding (mMol/kg) and crude-protein percentage in high-lysine (squares) and normal-lysine (dots) barley segregants from Fig. 6.

consequently be used as an estimation of KP. The correlation between mMol DBC/kg and mMol lysine/kg (Fig. 10) points out the remarkable fact that DBC related to lysine is lower for the high-lysine segregants than for the normal plants (MUNCK 1970a). Still, DBC/kg at different levels of KP is higher for the high-lysine segregants than the normal segregates (Fig. 11) owing to an in toto increase in the basic amino acids (lysine, histidine and arginine). Lysine, histidine and arginine are highly positively correlated with one another both in populations segregating for the lys gene (MUNCK 1970a) and in other barley materials (MUNCK et al. 1971). This fact consti-

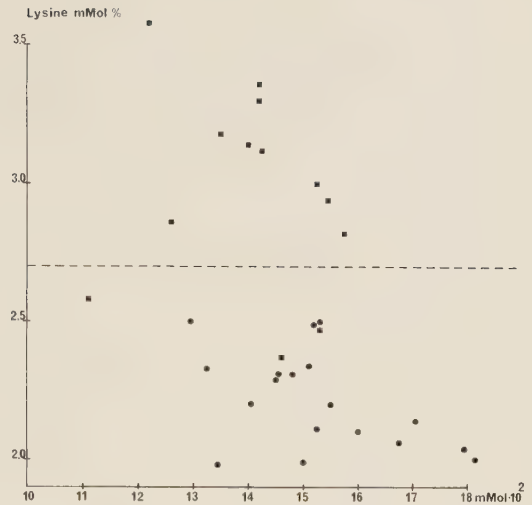


Fig. 12. Segregation of lysine based on single-seed analyses from a heterozygous F_1 plant from the cross Hiproly $\times$ Sv 66303. Lysine is related to the molar sum of 18 analysed amino acids as an estimation of crude-protein content according to Fig. 9.

tutes the empirical rationale for the use of the DBC method in screening for lysine.

To investigate the correlation between meal morphology and amino-acid composition, 100 F_2 seeds from an F_1 plant (Hiproly $\times$ normal) were screened for meal morphology (MUNCK 1970a) with the scratch technique (MUNCK et al. 1970). The experiment was performed as a blind trial with 20 seeds of each parent intermixed. There was a 1:3.2 segregation with respect to the adherence character to normal, which is not significantly different from a ratio of 1:3 (MUNCK 1970a). Thirty-one F_2 seeds representing different morphological classes were checked with amino-acid analyses. The lysine to arginine molar ratio was characteristically 0.84–0.90 in normal seeds and 0.95–1.04 in the starch protein-adherence class. One error in morphological classification was found in the F_2 seeds and one in Hiproly. The segregation in lysine (mMol %) for the 31 seeds that were analysed for amino acid is demonstrated in Fig. 12 and related to the molar sum of the amino acids as a representation for KP content. It is seen that the latter figure varies almost 100 % and that lysine (mMol %) is negatively correlated to the molar sum ("protein") on both the high-lysine and the

normal levels. Hence, it is evident that the recessive lys gene in its triploid state in the endosperm is expressed irrespectively of the heterozygous (Lys lys) constitution of the mother plant.

The pedigree analysis of the Hiproly crosses shall now be considered (Table 23). The material was divided into classes with regard to the amino-acid composition in F_2 plants, DBC in F_4 rows and morphology in the F_3 and F_5 seeds. DBC, KP, seed weight and coarse-meal percentages are listed for the various classes from rows in the F_4 generation. Classes Nos. 1 and 9 (Table 23) represent lines manifesting normal (N) or high (H) lysine, DBC:KP, or morphological characters in all the generations analysed. Class No. 9 is higher in DBC:KP, KP and coarse meal percentage and is considerably lower in seed weight compared with class No. 1 which displays normal (N) characteristics.

The corresponding naked (hull-less) classes Nos. 10 (N) and 11 (H), extracted from the whole material, are more extreme in DBC:KP than classes Nos. 1 and 9. Protein content is generally higher, seed weight and coarse meal reduced compared with the total material, most of which consists of hulled types. The naked-seed character is inherited independently of the lys gene (HAGBERG et al. 1970).

Class No. 8 represents high-lysine lines segregating from heterozygous F_2 plants having a normal phenotype with regard to amino-acid composition. These lines (8) have practically identical characteristics in the F_4 when compared with lines which were homozygous for the recessive lys gene in F_2 (9).

In the morphological screening of F_5 seeds, 1–2 seeds per line were analysed as a blind experiment with controls. Deviates were re-examined several times independently by two persons (in 5–10 seeds). Classes Nos. 6 and 7 represent lines that were high in DBC:KP in the F_4 and which were derived from normal (6) and high (7) F_2 plants. They displayed the starch-protein adherence character in the F_3 seed, but the F_5 seeds were morphologically normal, thus deviating from the pattern expected with regard to morphology. Seventeen lines from class 7 were checked by amino-acid analyses (Table 24). They did not differ from 15 high-lysine control lines. (The differences in the number of analysed samples in Tables 23–27 are due to the lack of material to perform all analyses.) The screening

analyses in the F_4 (Table 23) also confirm their high-lysine nature.

Likewise, classes Nos. 4 and 5 (Table 23) represent deviates with lower DBC:KP in the F_4 than expected. All the lines display the starch protein-adherence character in the F_3 and F_5 seeds. At least the lines (5) originating from high-lysine F_2 plants should be expected to be classified as high in DBC:KP in the F_4 . Amino-acid checks (Table 24) verify the correctness of this assumption. The low DBC in spite of high lysine/16 g N may be explained by a higher percentage of coarse meal in selections 4 and 5. Some of these lines were retested in F_5 in 1971 with respect to DBC:KP and compared with high-lysine control lines and with the morphological deviates (Table 25). The low DBC values in the F_4 were not repeated in the F_5 . The mill was changed between the two years. Milling factors are likely to contribute to the low DBC values in classes 4 and 5 (Table 23), although particle size should have been checked in the F_5 to allow a more definitive conclusion.

Class No. 3 in Table 23 represents possible recombinants combining high DBC:KP with normal morphology, and class 2 combines the adherence morphology, with otherwise normal characteristics. These lines were not verified further with respect to amino-acid composition.

In Table 26, correlation coefficients (r) between the characters studied in the F_4 material (Table 23) are given for the normal (Table 23:1) and the high-lysine classes (23:9), as well as for the corresponding naked lines (23:10 and 23:11) extracted from this material. The negative correlation coefficients for the KP–DBC:KP relationship (26:1) are about -0.90 in the normal material and lower in the high-lysine lines (-0.62), especially with regard to the naked material (-0.40). Neither seed weight nor meal grading (coarse meal %) (26:2–4) affected the KP–DBC:KP relationship. The lower correlation coefficients in the high-lysine material indicate a higher variability of BAA than in the normal lines. Seed weight is not significantly correlated with DBC:KP (26:7–8). There is a weak tendency for a negative correlation in seed weight–KP (26:5–6) for naked normal seeds and for the high-lysine selections. Seed weight tends to be negatively correlated to meal grading (26:10), whereas the opposite is the case for the high-lysine lines. This effect could be caused by a

Table 23. Characteristics* of pedigrees from four Hiproly crosses

Selection	Amino-acid composition (F ₂ yield)	DBC (F ₄ yield)	Morphology (F ₃ seeds)	Morphology (F ₅ seeds)	Lines in F ₅			Protein (%)	Seed weight (g)	Coarse meal (%)
					n	DBC/protein (μ mol/600 mg N × 6.25)	DBC**			
Whole material										
1.	N	N	N	N	141	671 ± 33	- 17	13.1 ± 1.9	40.1 ± 4.1	47.0 ± 1.9
2.	N	N	N H	H N	13	684 ± 38	- 8	12.9 ± 2.2	38.6 ± 4.3	49.6 ± 2.2
3.	N	H	N	N	15	675 ± 39	+ 16	15.0 ± 2.0	35.7 ± 4.0	47.5 ± 4.6
4.	N	N	H	H	7	699 ± 53	+ 7	12.9 ± 1.1	36.0 ± 2.5	62.6 ± 4.5
5.	H	N	H	H	12	657 ± 17	- 13	14.3 ± 0.8	34.9 ± 3.7	61.6 ± 5.6
6.	N	H	H	N	7	685 ± 19	+ 24	14.9 ± 1.4	33.3 ± 3.2	53.3 ± 5.5
7.	H	H	H	N	16	695 ± 21	+ 16	13.8 ± 1.5	34.0 ± 3.4	53.4 ± 2.8
8.	N	H	H	H	33	690 ± 32	+ 23	14.5 ± 2.1	33.1 ± 3.5	56.8 ± 7.5
9.	H	H	H	H	184	690 ± 20	+ 23	14.5 ± 1.4	32.7 ± 3.8	56.3 ± 5.8
Naked										
10.	N	N	N	N	19	644 ± 28	- 21	14.7 ± 1.8	37.3 ± 4.1	45.7 ± 3.4
11.	H	H	H	H	35	681 ± 36	+ 30	15.5 ± 1.2	32.2 ± 3.6	54.6 ± 6.5

* N = normal category, H = high lysine, high DBC or marked starch-protein adherence category

** Distance to regression line DBC (μ mol/600 mg N $\times$ 6.25) for category division

Table 24. Amino-acid composition (g/16 g N) of selected high-lysine lines in F₄

	Control lines (n = 15) [9]*	Low DBC deviates (n = 14) [5]*	Starch-protein adherence deviates (n = 17) [7]*
Lys	3.99 ± 0.17	4.00 ± 0.15	4.07 ± 0.15
His	2.13 ± 0.06	2.17 ± 0.08	2.18 ± 0.09
Arg	4.77 ± 0.16	4.73 ± 0.20	4.80 ± 0.12
Asp	6.31 ± 0.29	6.22 ± 0.29	6.37 ± 0.25
Ala	4.03 ± 0.16	4.07 ± 0.11	4.14 ± 0.13
NH ₃	3.02 ± 0.19	2.84 ± 0.26	3.10 ± 0.29
Glu	21.85 ± 0.88	21.73 ± 0.88	21.77 ± 1.19
Pro	10.59 ± 0.48	10.68 ± 0.55	10.42 ± 0.42
Met	1.62 ± 0.12	1.58 ± 0.09	1.57 ± 0.12
DBC/kg	167.4 ± 13.8	155.7 ± 5.4	159.2 ± 12.1
DBC/g protein	1.14 ± 0.03	1.09 ± 0.03	1.16 ± 0.03
Protein	14.74 ± 1.46	14.34 ± 0.75	13.82 ± 1.43
Σ mmol/kg**	1307 ± 136	1257 ± 70	1238 ± 142

* Selections from Table 23 ** 18 amino acids

Table 25. The protein—DBC relationship of different high-lysine lines selected in 1970 for deviating DBC and starch-protein adherence and grown in 1970 and 1971

Selections (Table 23)	Year	n	Crude protein (%)	DBC*	r	b	a
9. Control (high DBC), marked starch-protein adherence	1970	12	14.9 ± 1.5	682 ± 18	—0.66*	8.4	808
	1971	12	15.1 ± 1.9	687 ± 35	—0.77**	13.9	898
5. High DBC, normal starch-protein adherence	1970	14	13.3 ± 1.0	701 ± 14	—0.90***	12.3	865
	1971	14	13.8 ± 1.3	716 ± 16	—0.78**(*)	9.9	852
7. Low DBC, marked starch-protein adherence	1970	7	14.0 ± 0.8	668 ± 8	—0.82*	7.9	779
	1971	7	14.5 ± 1.6	697 ± 33	—0.97***	20.9	999

* DBC μ mol/600 mg N × 6.25

different milling performance owing to the starch protein-adherence character. Meal grading is generally weakly negatively correlated (26:10) with KP.

In Table 24 the average amino-acid composition per 16 g N of 15 control lines (from 23:9) in the F₄, representing different high-lysine F₂ plants is compared with mean patterns of the 14 DBC (23:5) and the 17 morphological (23:7) deviates. The differences are not significant. The standard deviations of the amino acids are of the same magnitude. Nevertheless, there are large differences with respect to the correlation coef-

ficients for amino acids/16 g N—KP and amino acids—DBC:KP between the three selections (Table 27). In normal barley lysine in percentage of protein is negatively correlated with the KP content (MUNCK 1970a). The corresponding coefficient for high-lysine segregants display much lower correlation coefficient. In Table 27 (F₄), it is found that the control lines and the DBC deviates do not show a negative correlation between lysine g/16 g N—KP ($r=0.0$ and 0.1 resp.), which is in sharp contrast to the morphological deviates, which do not show the starch-protein adherence character ($r=-0.8$). Similar differ-

Table 26. Correlations (r) between different characters in F₄ material segregating for the high-lysine gene

	Normal barley		High-lysine barley	
	(n = 141)	naked seed (n = 19)	(n = 184)	naked seed (n = 35)
<i>Protein — DBC/g protein</i>				
1. Simple correlation	−0.91	−0.89	−0.62	−0.40
2. Seed weight excluded	−0.91	−0.89	−0.65	−0.42
3. Meal-grading excluded	−0.89	−0.89	−0.62	−0.27
4. 2 and 3 excluded	−0.90	−0.90	−0.64	−0.34
<i>Seed weight — protein</i>				
5. Simple correlation	−0.02	−0.25	−0.32	−0.47
6. Meal-grading excluded	−0.05	−0.43	−0.27	−0.34
<i>Seed weight — DBC/g protein</i>				
7. Simple correlation	−0.14	0.16	0.03	0.05
8. Meal-grading excluded	−0.07	0.24	0.00	−0.15
<i>Seed weight — meal-grading</i>				
9. Simple correlation	−0.21	−0.41	0.24	0.43
<i>Meal-grading — protein</i>				
10. Simple correlation	−0.33	−0.30	−0.25	−0.43
<i>Meal-grading — DBC/g protein</i>				
11. Simple correlation	0.38	0.14	0.12	0.40

Table 27. Correlation coefficients (r) between amino acids (mol %), protein (%) and DBC (mmol) of selected high-lysine lines in F₄

	r with protein			r with DBC/g protein		
	Control lines (n = 15) [9]*	Low DBC deviates (n = 14) [5]*	Starch protein adherence deviates (n = 17) [7]*	Control lines (n = 15) [9]*	Low DBC deviates (n = 14) [5]*	Starch protein adherence deviates (n = 17) [7]*
Lys	0.0	0.1	−0.8	0.4	0.3	0.8
His	0.0	−0.4	−0.4	0.2	0.6	0.3
Arg	0.3	−0.1	−0.7	0.1	0.2	0.6
Asp	−0.4	0.0	−0.1	0.6	0.0	0.2
Ala	−0.7	0.0	−0.7	0.8	0.2	0.7
NH ₃	0.5	−0.1	−0.1	−0.6	−0.1	0.1
Glu	−0.4	0.1	0.7	0.2	0.0	−0.7
Pro	0.2	0.0	0.5	−0.2	0.1	−0.5
Met	0.6	0.6	0.0	−0.3	−0.4	−0.1
DBC/kg	1.0	0.8	1.0	−0.5	−0.2	−0.9
DBC/g protein	−0.7	−0.8	−0.9	−0.7	−0.7	−0.9
Σ mmol/kg**	1.0	0.9	1.0	−0.7	−0.7	−0.9

* Selections from Table 24 ** 18 amino acids

Table 28. Amino-acid composition of barley lines in F₂, F₄ and F₅ (Hiproly × Normal)

Amino acids (g/16 g N)	F ₂ plants in pots outdoors 1969	Selected line from F ₃		Controls	
		F ₄ plants in field 1970	F ₅ plots in field 1971	1970	1971
	Normal segregant 69/21257			Normal CI 4362	
Lys	3.39	3.55	—	2.95	3.10
Asp	6.57	6.44	—	4.87	5.56
Glu	26.35	24.12	—	25.22	25.54
Protein (N × 6.25)	14.8	13.9	—	17.20	14.30
	High-lysine modified segregant 69/21301				
Lys	3.91	3.92 ± 0.12 (n = 7)	3.97		
Asp	6.64	6.84 ± 0.19	6.26		
Glu	24.80	23.02 ± 0.72	22.66		
Protein (N × 6.25)	16.2	13.4 ± 0.7	13.2		
	High-lysine segregant 69/21909			Hiproly CI 3947	
Lys	4.22	4.51 ± 0.02 (n = 2)	4.30	3.98	4.19
Asp	7.18	8.04 ± 0.05	7.01	6.33	6.80
Glu	24.72	20.54 ± 0.20	21.60	21.69	22.73
Protein (N × 6.25)	14.5	12.1 ± 1.2	13.0	17.7	16.4

ences exist with respect to arginine, glutamic acid, proline and alanine.

The correlation coefficients in the relationship amino acids g/16 g N—DBC:KP (Table 27) display differences analogous with the correlations with protein, but with opposite sign.

As was previously pointed out, two of the four crosses between Hiproly × normal gave segregation ratios (regarding DBC:KP—KP) in the F₂ that differed significantly from a 1 to 3 ratio (MUNCK et al. 1970). These combinations displayed deficits of recessives. When F₄ families representing different F₂ plants from one of these combinations were analysed with DBC, related plants tended to cluster together on different levels in the DBC:KP—KP relationship. These differences were verified by amino-acid analyses (MUNCK 1972a). It is thus evident that the genetic background plays a decisive role for the expression of the lys character in the endosperm. In Table 28, three lines—one normal, one high-lysine, and one modified high-lysine segregant—are compared in the F₂, F₄, and F₅ generations with Hiproly and the normal sister line (CI 4362) as controls. The three lysine levels—normal, high, and modified—are clearly demonstrated with due consideration to the different KP levels in

the different generations and years. Asparagine increases and glutamic acid decreases in protein with improved lysine content.

To estimate the effect of the lys gene on yield capacity, normal segregants were compared with high-lysine ones in the F₃ in growth chambers at 14 and 24 hours of day length (HAGBERG et al. 1970). In Table 29 the cross Hiproly × Sv 66350 is examined. Ear-emergence date is approximately 1 month earlier at 24 hours of day length when compared with 14 hours of day length. Hiproly is 3 weeks earlier than Sv 66350 at 14 hours but almost 4 weeks later at 24 hours of day length. The average differences in earliness between the normal and high-lysine segregants are small when comparing similar conditions. Grains per plant and yield are strongly negatively associated with the long day and the consequently short vegetation period. Hiproly was most strongly negatively affected. An exception is Sv 66350, which reacts positively towards a long day. There are small differences in number of grains per plant between the normal and high-lysine segregants, the 1000-grain weight being, however, generally lower in the high-lysine lines. Hence, yield is moderately reduced in the high-lysine segregants compared with the normal ones. Straw

Table 29. Agronomic characteristics of parents and F_3 families of Hiproly $\times$ Sv 66350 grown at 14 and 24 hours day length

Origin	Protein	Day length (hr)	Plants (n)	Ear emergence (date)	Grains per plant (n)	1000 grain weight (g)	Grain yield per plant (g)
Sv 66350	Normal	14	16	9 Mar.	103	22.4	2.3
"	Normal	24	16	1 Jan.	79	37.7	3.0
F_3 families	Normal	14	40	26 Feb.	73	40.9	3.0
"	Normal	24	36	29 Jan.	55	46.9	2.5
"	High-lysine	14	66	1 Mar.	78	34.9	2.7
"	High-lysine	24	62	1 Feb.	59	38.9	2.2
Hiproly	High-lysine	14	3	16 Feb.	83	36.8	3.1
"	High-lysine	24	2	24 Jan.	11	49.3	0.5

Mean of each population is calculated as average of investigated families

length and shoots per plant are not affected by the lys gene, whereas crude-protein content is elevated by about 1 % (MUNCK et al. 1970), probably owing to the decreased seed weight. The shrivelled seed type could, however, be modified to smooth seeds and the 1000-grain weight could likewise be increased without losing the expression of the lys gene (HAGBERG et al. 1970).

The yield of the best F_4 line in field trials is about 70 % of the standard variety Ingrid. Twelve yield plots, out of 128 high-lysine back-cross lines to the commercial variety Mona, displayed more than 90 % in yield, six more than 95 % and two more than 100 % compared with a Mona control in each block of nine back-cross lines (KARLSSON 1972 pers. comm.).

B. Discussion

Because of the high content of essential amino acids in the embryo, the relative size of embryo and endosperm might affect the amino-acid composition of the whole seed. This is especially valid in maize where the germ constitutes 10–20 % of the whole seed (BALINT 1970), compared with 3.5–4.5 % (embryo+scutellum) in barley (MUNCK 1970a). NELSON (1969a,b) demonstrated that the opaque-2 (o_2) and floury-2 (fl_2) genes change the amino-acid composition of the endosperm, but not that of the embryo. The lys gene in barley also expressed itself exclusively in the endosperm (MUNCK 1970a). The amino-acid pattern in the embryo, however, varied

more in a barley material (MUNCK 1970a) than in maize (NELSON 1969b). The question if the embryo+scutellum contains proteins of storage character that could be genetically modified in amino-acid composition could not, however, be answered in the limited barley investigation (MUNCK 1970a). The leguminous seed having an analogous morphological origin has, however, developed abundant, storage proteins that are rich in arginine, agreeing with the high-arginine content of the monocotyledonous embryo+scutellum. Selection for high protein content in the legume *Vicia faba* results in a nitrogen increase that is to 43 % due to arginine nitrogen (MUNCK et al. 1972b), indicating a predominant enhancement of the arginine-rich storage proteins.

When comparing the amino-acid composition (Table 30) of the high-lysine mutants in maize and barley, the whole-seed level displays lower lysine g/16 g N values for lys barley as compared with floury-2 and opaque-2 (maize data from NELSON 1969b). In o_2 and fl_2 maize, lysine, histidine and arginine are increased and serine, glutamic acid, proline, alanine, isoleucine, leucine, tyrosine and phenylalanine are decreased when compared with normal maize. The two mutants differ mainly in aspartic acid and methionine, which are higher in fl_2 , and glycine and cystine, which are more abundant in o_2 . The changes in barley due to the lys gene is less dramatic than with the high-lysine genes in maize. The changes due to the lys gene in barley resemble more that of fl_2 in relation to normal maize, lysine, aspartic acid and methionine being

Table 30. Comparison of the effect of the Opaque-2 and Floury-2 genes in maize and the high-lysine gene in barley (whole seeds)

	Normal* maize g/16 g N	Opaque-2* (normal maize = 100)	Floury-2*	Normal** barley g/16 g N	High lysine** barley (normal = 100)	High-lysine** barley (floury-2 = 100)
Lys	3.0	167	160	3.4	123	86
His	2.6	135	112	2.1	103	75
NH ₃	—	—	—	3.6	89	—
Arg	4.9	147	129	4.6	107	77
Asp	9.2	96	114	6.0	114	65
Tre	4.1	93	100	3.4	106	88
Ser	5.6	84	93	4.3	102	85
Glu	22.6	76	82	26.8	89	129
Pro	9.6	87	92	12.6	90	128
Gly	4.7	109	100	3.6	107	83
Ala	9.2	72	87	3.8	115	54
Cys	1.7	118	94	1.1	81	—
Val	5.7	91	100	4.8	110	92
Met	—	92***	112***	1.2	121	—
Ileu	4.2	81	95	3.7	105	98
Leu	14.6	64	82	6.7	106	59
Tyr	5.2	81	88	2.8	101	61
Phe	5.8	76	90	5.9	100	113
Try	0.7	185	—	—	—	—
Protein	9.0	117	189	15.7	108	101

* NELSON (1969b)

** Mean of 35 normal and 27 high-lysine plants from a segregating F₂ population. Non-oxidized samples: Cys and Met absolute values not reliable

*** Approximation: normal maize = 2.3 g/16 g N.

increased, cystine, glutamic acid and proline decreased. The amino-acid pattern of normal barley is more favourable from a nutritional point of view than that of normal maize, especially with regard to lysine, cystine and tryptophane and the leucine : isoleucine balance. Tryptophane is 0.7 g/16 g N in normal maize (Table 30) and is greatly improved in the mutant, whereas in barley the tryptophane content is 1.3 g/16 g N and is not changed in high-lysine barley (EGGUM pers comm.).

The result from a comparison of endosperm amino-acid composition in the maize and barley mutants (Table 31) is very different from that seen when whole seeds are studied (Table 30). This is due to the differences in embryo to endosperm ratio discussed above. It is observed that Hiproly endosperm is higher in lysine compared with both o₂ and fl₂. The maize mutants are especially rich in aspartic acid, alanine and leucine, whereas Hiproly has higher levels of glutamic acid, proline and phenylalanine. Thus, endosperm and embryo amino-acid composition

should be compared separately when discussing phylogenetic differences in protein evolution of cereal seeds.

Another pathway to improve the lysine content is to increase the number of aleurone layers that are rich in nutritious proteins. Multilayered aleurone have been observed in maize by WOLF et al. (1969). Barley normally has 2 to 3 layers. The proportions of aleurone material in the seed could also be raised by increasing the surface area of the seed. This can be obtained in maize by utilization of the mutant genes determining the pistillate inflorescence (NELSON 1970), leading to a greatly increased number of smaller seeds.

It is interesting to note that all three high-lysine mutants in cereals extensively analysed (o₂, fl₂ and lys) to date are associated with a changed morphology—in the opaque-2 case the seed is smaller and lighter as compared with the isogenic mother line (NELSON 1966). Owing to genetic background, NELSON further points out that density may vary from 4 to 40 % as compared with normal plants in different o₂

lines. It should thus be possible to select dense o_2 lines. The soft character of the o_2 lines results in a higher water content in the grain and a greater tendency for the kernel to crack (ALEXANDER et al. 1969). Problems are thus likely to rise in harvesting and storage and, likewise human acceptability is likely to be affected by the milky appearance of the o_2 and fl_2 kernels. The great advantage with the association of the morphological character to the change in amino-acid composition in o_2 and fl_2 is the simplified plant-breeding techniques which make chemical screening analyses unnecessary. A growing interest to produce non-opaque o_2 lines in maize for improved grain properties has thus developed. PAEZ et al. (1969) found modified o_2 kernels that were $\frac{1}{2}$ opaque : $\frac{1}{2}$ translucent in segregating progenies. The lysine content did not differ from that of opaque kernels from the same ear; on the other hand, translucent and opaque fractions from the same o_2 seed were not changed in lysine, which has been confirmed by BÁLINT et al. (1970). Later almost translucent high-lysine seeds carrying the o_2 gene have been obtained (VILLEGAS 1972).

The lys gene in barley is, in contrast to the soft opaque-2 in maize, associated with a hard endosperm character that could also be modified as was previously mentioned. The question now arises if the phenotypic effects on morphology in each of barley and maize are due to separate genes or if they are pleiotropic with the same causal chain as the amino acid trait. The amino-acid change in the endosperm of o_2 , fl_2 , and lys genes is due to a major alteration in the synthesis of already existing proteins, favouring some of the lysine-rich ones and depressing those displaying a low nutritional value (NELSON 1969b, 1970; MUNCK et al. 1969; MUNCK 1972a, b). These effects will be discussed in more detail in Chapter IV.

LAMBERT and ALEXANDER (1968) showed that the spontaneous mutation rate in the opaque-2 locus was about one per 300,000 female gametes. The mutation rates differed with regard to the genetic background. BÁLINT et al. (1970), in mutation experiments, found a mutation rate of 1:6225 and 1:11846 in two strains. It was found that some of these mutants also exhibited a change in the amino-acid pattern similar to that in the original o_2 mutation. In addition, a large number of opaque mutants were found that were

Table 31. Comparison of endosperm amino-acid composition (g/16 g N) of Hiproly and maize Opaque-2 and Floury-2

	Barley Hiproly	Maize genotypes (NELSON 1969b)	
		W64Ao ₂ Opaque-2	fl_2 Floury-2
Lys	4.0	3.7	3.3
His	2.1	3.2	2.2
NH ₃	3.3	—	—
Arg	4.4	5.2	4.5
Asp	6.2	10.8	8.1
Tre	3.5	3.7	3.3
Ser	4.6	4.8	4.8
Glu	24.0	19.8	19.1
Pro	12.0	8.6	8.3
Gly	3.7	4.7	3.7
Ala	4.2	7.2	8.0
Cys	1.7	—	1.8
Val	5.3	5.3	5.2
Met	2.0	1.8	3.2
Ileu	3.9	3.9	4.0
Leu	7.0	11.6	13.3
Tyr	2.9	3.9	4.5
Phe	6.0	4.9	5.1
Protein	17.2	11.1	13.6

not associated with a change in the amino-acid composition. Thus, in the opaque-2 case, it seems appropriate to conclude that both the morphological and the amino-acid changes may be affected by one mutation event in a very small part of the seventh chromosome of maize. Nevertheless, the opaque character might be modified by manipulation of the genetic background without affecting the amino-acid composition. It seems less likely that the modified effect is due to crossing-over between one morphological and one protein-regulating gene.

In the case of the lys gene in barley, an analogous condition seems to prevail, although more evidence has to be collected for verifying proof. Out of 235 high-lysine recombinants, 17 were found to be normal in morphology, the others possessing the peculiar starch protein-adherence character. As shown in Chapter IV, there is no doubt that this morphological trait is based on true structural modifications, the effects of which, however, are difficult to interpret. The endosperm is characteristically dense and flinty when cut with a knife, although more soft types exist that still exhibit the starch-protein adherence charac-

ter. Parts of the endosperm could also be modified to a more mealy character, which observation is in accordance with the opaque-2 case. From our investigations in barley, we cannot conclude that all 17 high-lysine morphological deviates are fully modified throughout the whole seed. Starch-protein adherence barley lines were found (e.g. Monte Cristo) which are microscopically similar to the one which is associated with the lys gene. Their amino-acid patterns were, however, normal. It is obvious that many genetically dependent, previously neglected characters influence the morphological and ultrastructural properties of the barley endosperm.

The starch-protein adherence character found in Hiproly does not seem to influence the amino-acid composition in the endosperm directly (Table 24). It does, however, affect the correlation coefficients between different amino acids and protein (Table 27), e.g. so that the usual negative correlation between lys g/16 g N—crude protein now is insignificant. *Thus, it is possible to select for a changed response with regard to the relationship between protein and the essential amino acids.* As was pointed out earlier, oats do not normally exhibit a negative lysine g/16 g N—crude protein correlation. ROBINSON and SAGEMANN (1968) studied South African and Australian wheat varieties grown under irrigation conditions in North-Western Australia. They could not find any significant relationship in lysine g/16 g N—crude protein. This could be attributed either to growing conditions or to variety. Normally, most of the essential amino acids in wheat protein are negatively correlated with total crude-protein content.

It is generally agreed that crude-protein content in cereals is strongly susceptible to environmental influences, which raises problems of identification of genetic effects (JOHNSON et al. 1969). In the screening work in Sweden and Denmark for high-lysine barley by MUNCK et al. (1970), TOFT-VIUF (1972) and DOLL (1971), *the most striking result is that even very small improvements, as 10 % in lysine g/16 g N, are found to be environmentally stable in relation to a standard material.* TOFT-VIUF (1972) screened a barley sortiment with an amide analysis technique utilizing the negative correlation between lysine g/16 g N—amide percentage of N as an estimation of lysine. One barley variety with a 10 % increased lysine content of protein compared with normal

varieties was found. This line was higher also in DBC : KP according to TOFT-VIUF. These results could be verified on material grown and analysed in Svalöf (MUNCK 1972a).

In the pedigree material presented for studies of the lys gene, analyses in the F_4 convincingly demonstrate that single plants from the same family coincide on different DBC and lysine levels with regard to the DBC : KP—KP and the lysine g/16 g N—KP regressions, respectively as demonstrated in Table 28. With regard to amino acids lysine g/16 g N and related characters therefore, seem to be very stable in different environments and also to display a much higher heritability than the crude-protein character. This is in accordance with the nitrogen regulation principle of BISHOP (1928, 1930) who also stated that the relationship between the Osborne protein fractions and the crude-protein level was dependent on variety. It thus seems appropriate to utilize also minor genes affecting the balance between lysine-rich and lysine-poor protein sub-fractions of the cereal endosperm. Due consideration has to be taken to define whether the improvements are due to gross morphological reorganization in changed embryo : endosperm and aleurone: endosperm ratios or to an alteration of endosperm amino acid composition. Different approaches could then be used in breeding for an altered amino-acid composition. Similar results to that of the modification of amino-acid composition in barley have been discussed for maize by NELSON (1969b, 1970), whereas data from wheat seem to be less conclusive (MATTERN et al. 1970). However, FAJERSON (cit. MOSSBERG 1965) indicated a different ratio of DBC:KP between two winter wheat varieties grown 4 years at seven nitrogen levels. No amino-acid analyses were made. A dominant suppressor gene acting on the expression of the opaque-2 gene in maize was found by POLLACSEK (1970). The modified opaque-2 kernels, however, that are homozygous for the o_2 gene are practically normal in texture and in lysine content.

2. Gene-environment interaction

In order to optimize environment and plant with respect to production of edible parts and nutrients, the plant breeder needs further insight

into the conditions of plant development and growth as obtained with laboratory techniques and in field trials. In the following discussion, two rather theoretical experiments will be considered with plants grown in pots.

In the first trial (Fig. 13 and 14), four barley varieties were grown at varying temperatures and day lengths in a phytotron according to DORMLING et al. (1966, 1969). Nutrients were given in increasing amounts (DORMLING et al. 1966, 1969) differences in growth, however, not being considered when administering the fertilizer levels. In the second experiment (Fig. 15—18, Table 32), three barley varieties were grown outdoors at different N, P and K levels under a plastic roof with no walls. Water was equally administered to all pots. In the first experiment, plants were grown in sand; in the second, in a sand-perlite mixture.

These two experiments were grown under conditions very remote from those in the field and the results should be interpreted with these facts in mind. The design of the experiments was set up mainly to study the plasticity of different genotypes in extreme conditions.

A. The effect of different photo- and thermal periods in relation to protein and lysine formation in barley¹

In this trial (Fig. 13, 14), the previously discussed cultivar Hiproly (H) and its normal sister line (S) were compared with the early X-ray mutant Mari (M) and its parental variety Bonus (B). Mari barley, or early-a⁸, was isolated in 1950 after mutagenic treatment and approved for release by the Swedish Board of Agriculture in 1960 as a commercial variety (GUSTAFSSON et al. 1960). This variety thus differs in one recessive gene from the parental variety Bonus. The gene increases photoperiodic insensitivity with regard to ear formation, heading capacity and kernel production (DORMLING et al. 1966). Under extremely long-day conditions (24 hours of light), there are no differences in heading time between Mari and Bonus; but with a moderate long-day photoperiod (16 hours of light), the differences in heading time are more than 3 weeks. The relative photo- and thermal period insensitivity of Mari is pleiotropically combined with earliness, low stature, lodging resistance and high yield. Mari is, however, inferior to its parental variety in yield under the climatic conditions for which the latter variety has been bred. In the experi-

ment represented in Fig. 13, the plants have been grown at 8, 16, 20, and 24 hours of day length at 20/15°C, 20/10°C, and 15/10°C (day/night temperatures). Seeds per plant, seed weight and yield per plant are studied. The previously cited results regarding Mari (M) and Bonus (B) are verified. Mari alone produces seeds at 8 hours of day length. A medium day length of 16 hours produces at the higher temperature 20/15°C and 20/10°C a low number of seeds per plant and a decreased yield in Bonus when compared with Mari. At 16 hours, 20/15°C seed size is depressed much more for Bonus than for Mari. The low temperature 15/10°C gives higher yields than the higher ones, especially for Bonus.

In the range 16–24 hours of day length, Hiproly (H) and its sister line (S) seem to be even more temperature and day-length insensitive than Mari. The number of seeds per plant and yield are considerably lower for Hiproly and the sister line. The former being inferior, especially with regard to seed weight. Climatic responses of the two lines, which are considered to be more or less isogenic, are almost identical. A day length of 16–20 hours at 15/10°C gives maximum yields.

Number of days for the first flag leaf to emerge were 40.7 ± 2.4 , 42.4 ± 1.0 , 53.4 ± 1.9 , and 110.4 ± 2.6 at 15/10° and 24, 20, 16, and 8 hours of day length, respectively, for Bonus. The corresponding figures for Mari were 39.0 ± 3.0 , 36.0 ± 1.0 , 39.4 ± 1.7 , 55.9 ± 1.3 ; for Hiproly, 40.9 ± 1.2 , 41.3 ± 2.4 , 52.6 ± 1.9 , 99.4 ± 1.1 ; and finally for the sister line, 42.7 ± 0.5 , 41.4 ± 0.5 , 53.7 ± 1.0 , 102.1 ± 2.4 (DORMLING pers. comm.) Hiproly generally tended to be a few days earlier than the sister line, both varieties resembling Bonus more than Mari at 15/10°C. Corresponding varietal differences were obtained for the maturation time for the first spike. At temperatures of 20/15°C and 20/10°C, the Hiproly and its sister line resembled Mari more in earliness than Bonus, indicating a specific temperature response for the two Ethiopian varieties. The maturation time was considerably reduced here, especially for Hiproly at 24 hours of day length.

The chemical composition of the seed is indicated in Fig. 14 at growth conditions of 20/15°C (8, 16, 20, 24 hours of day length), 15/10°C (8, 16, 20, 24 hours of day length and at 16 hours of

¹ See MUNCK et al. 1972 a.

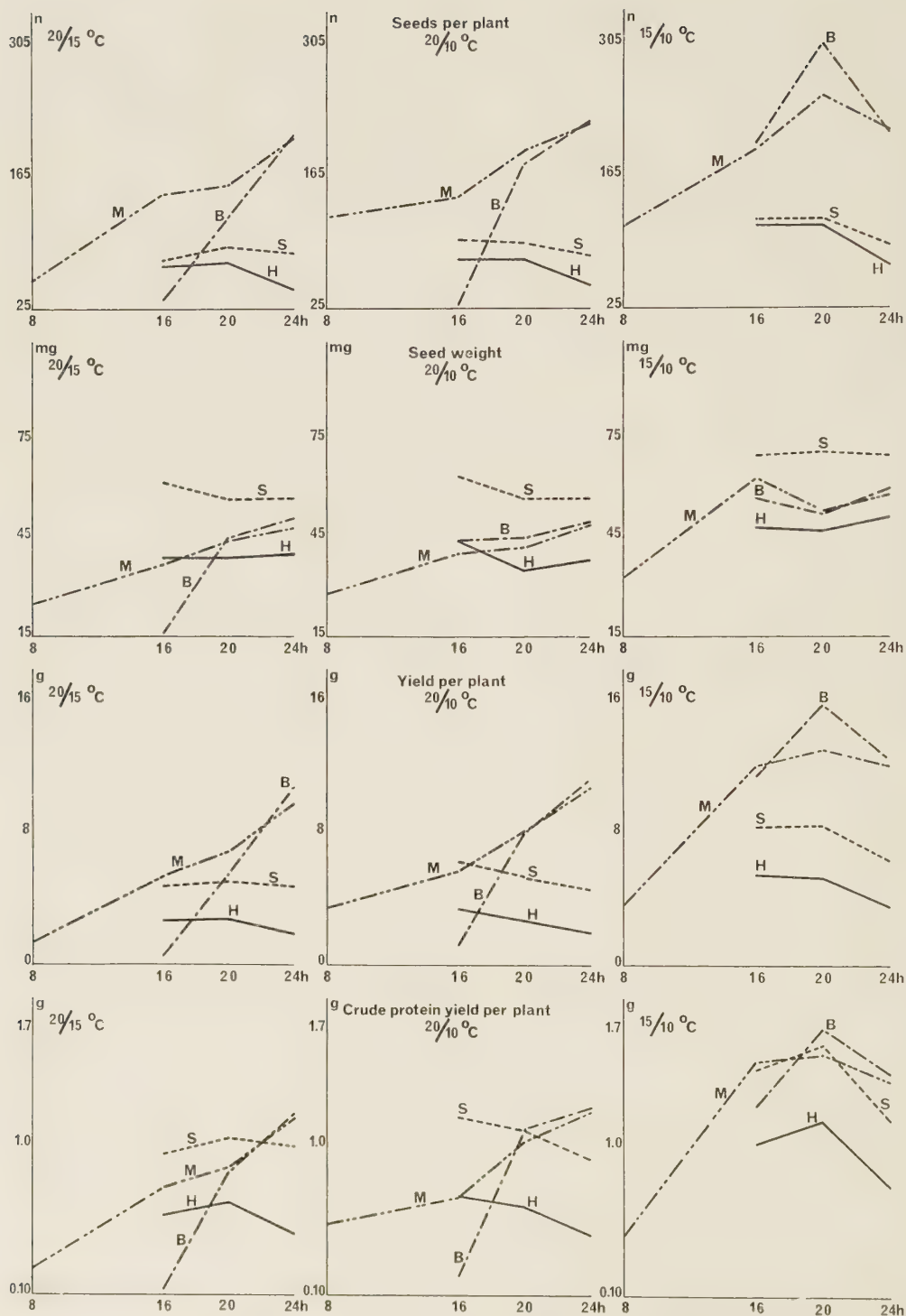


Fig. 13. Phytotron experiment showing barley varieties Hiproly (H), CI 4362 or Hiproly sister line (S), Bonus (B), and the early mutant Mari (M) from Bonus at different day lengths (8, 16, 20, and 24 h) and day/night temperatures (15/10, 20/10, and 20/15°C). Number of seeds per plant (g), seed weight (mg), yield per plant (g), and crude-protein yield per plant (g) are shown for the different treatments.

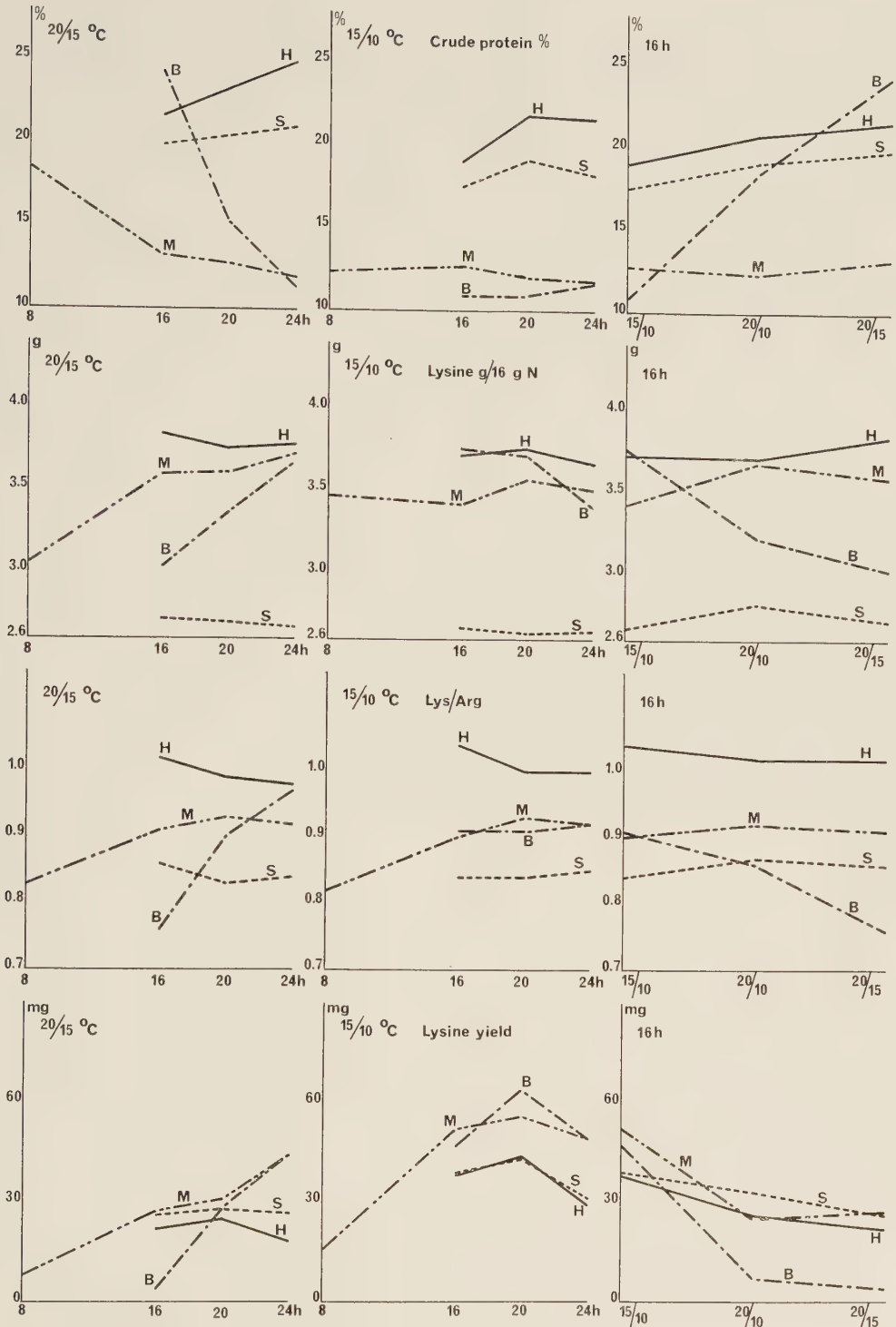


Fig. 14. Crude protein (%), lysine g/16 g N, molar lysine: arginine ratio and lysine yield (mg) from phyto-tron experiment in Fig. 13.

day length (15/10°C, 20/10°C, 20/15°C). As regards the crude-protein percentage, Hiproly and the sister line were generally higher when compared with Bonus and Mari. The former lines are also much more constant in this character under the different climatic conditions. If crude protein percentage (Fig. 14) and seed weight are compared (Fig. 13), it is seen that a reduction in seed size results in an increase in crude-protein percentage, especially in Bonus. This increase is due to a reduction in starch synthesis under the adverse environmental conditions.

With regard to lysine g/16 g N (Fig. 14), Hiproly (about 3.7 %) is higher than the sister line (about 2.7 %), Mari and Bonus being intermediate. Also here, the constancy of the two Ethiopian varieties in the different environments is very well marked. When protein percentage increases, there is a tendency for Bonus and Mari to have a decreased lysine in the protein.

A high lys:arg molar ratio (Fig. 14) has been used earlier by MUNCK (1970a) to define the presence of the lys gene in segregating seeds and is regularly used in the plant-breeding work in Svalöf. It is therefore interesting to see how the stability of this character is influenced by climatic conditions. The lys:arg molar ratio is affected by temperature and day length especially at the extreme conditions. Nevertheless, there is a clear differentiation, Hiproly being higher than the sister line, with Mari and Bonus intermediate.

Yield in crude protein (Fig. 13) and yield in lysine are interactions between the agronomic characters in Fig. 13 and the chemical composition in Fig. 14. Owing to its high protein content, the sister line reached the level of crude-protein yield per plant of Mari and Bonus, Hiproly being inferior. In lysine production, the two Ethiopian lines were nearly identical but they were less productive than Bonus and Mari at 15/10°C and 16–24 hours of day length.

The phytotron trials prove that the lys gene is relatively independent of day length and temperature. The negative relationship lysine g/16 g N—KP is also confirmed in this trial (Fig. 14). The objection could be made that the plants have obtained equal amounts of fertilizer irrespective of vigour or poor growth. Plants growing slowly under certain climatic conditions are thus likely to receive an excess of minerals that could produce a high crude-protein content, especially

in the case of Bonus. This study could thus be regarded as an indirect fertilizer trial with temperature and day length as releasing mechanisms. This objection is valid, especially with regard to the chemical composition. Characters as early maturity and number of seeds are more reliable parameters in characterizing temperature and day-length responses of the different varieties. The relative insensitiveness of the mutant Mari to temperature and day length is well documented in field trials according to DORMLING *et al.* (1966).

The poor-yielding capacity of Hiproly barley also when compared with its sister line—confirmed in field trials in Sweden and the U.S.A.—raises the question whether the lys character may be included in high-yielding barley lines. As was pointed out in the previous discussion, seed size seems to be the critical character that needs further improvement. GUSTAFSSON *et al.* (1971) described a mutant (44/3), obtained by X-rays in 1939, having an extreme straw strength but displaying a low 1,000 kernel weight. After several crosses with the large-kernelled variety Birgitta, the small grain size was finally overcome and the result was the release of the commercial Svalöf variety Gunilla in 1970. Also mutants with depressed yield but with favourable quality characteristics should be worth considering in breeding programmes if the gene background can be adjusted to exclude the negative pleiotropic effects of the new gene. Such an operation might be difficult to achieve or it may even fail, but the plant breeder has no other choice than to believe that such an endeavour ought to be profitable. There are enough practical examples to support him.

Problems with regard to small seed size are also prevalent in opaque-2 maize breeding (SREERAMULU and BAUMAN 1970). Opaque phenotypes were 11 % lower in kernel weight and yield than their normal counterparts. Kernel number per ear was unaffected, whereas opaque lines had 11.4 % in crude protein compared with 10.6 % for the normal lines. There was no negative relationship between lysine and protein levels and yield, indicating good possibilities to obtain opaque-2 lines of high productivity.

B. The effects of different nitrogen, phosphorus, and potassium fertilizer levels in relation to nitrogen uptake and lysine synthesis in barley seeds

Seeds were sown in rows (10 plants/row) in wooden boxes. Sv 59135 (MUNCK 1964 a) — a high-yielding, low-protein barley line — was compared with 1 six-row and 1 two-row barley line (obtained from WIEBE, A. HAGBERG pers. comm.) being isogenic except for the Mendelian spike character as a result of repeated backcrossing. From each line and box, one or two rows were obtained. Fertilizer was administered from stock solutions composed as described in Table 32. Nutrients were supplied 1 to 3 times a week; each application was 10 ml. The standard conditions were 2N2, 2K2, 2P2 corresponding to 2×10 ml of the substrates N2, K2, and P2 described in Table 32, including 10 ml of each of solutions I—III containing the remaining minerals needed. Three series of dressings were arranged varying N, K, and P exchanging the concentration of the respective element in the standard dose 2N2, 2K2, 2P2 with N1, 3N1, N3, and 5N2; K1, 3K1, and 4K2; P1, 3P1, P2, and 5P2 according to the formulas in Table 32. Dressings were gradually diminished at heading and terminated, except for the highest doses 5N2, 4K2, 5P2, which were continued for 2 weeks after heading. Plants were harvested individually and were measured and weighed. Chemical analyses were performed on samples representing rows. There were two replication boxes in the standard treatment and in 3N1, 3K1, 3P1, N2, K2 and P2.

In Table 32, agronomic characters are described for the barley variety Sv 59135, together with the chemical composition of the seed (ash, P, K, Na, Fe, lysine g/16 g N, protein, and lysine yield), grown at variable N levels in perlite and in soil. Each figure represents means of two rows from the same box. At the 2N2 level results from two replicated boxes are compared, which show reasonable agreement except with regard to iron content.

In perlite, tillering and seed yield is greatly improved by increasing the nitrogen supply up to N2, which gives the yield optimum. Protein content is low (6.0–8.4 %) at these dressings and lysine g/16 g N is very high (4.52–4.06 g/16 g N). The doubling of the N dose from N2 to 2N2 also nearly doubles the protein content in the seed, whereas seed weight and grain yield decrease. The standard condition 2N2 has been chosen too high in this experiment if grain yield was to be optimized. Lysine content is reduced to 3.19 g and 2.58 g/16 g N for N2 and 5N2. Because of the use of NaNO_3 and NaH_2PO_4 in the nutrient medium, the Na ion, which is not essential for the plant, also accumulates in the seed. A deleterious effect of Na at the dosage levels 2N2 and 5N2 should not be overlooked. It is interesting that the highest dose, 5N2, apparently

reduces the Na content of the seed in spite of the increased Na supply.

The soil medium at the 2N2 dressing level could be compared with that of the perlite medium, yield being, however, lower due to an excess of nutrients. Potassium and especially sodium are reduced in seeds from plants grown in soil, which emphasizes the importance of the medium for ion accumulation. With regard to lysine and protein composition, similar effects could be shown in the soil as in the perlite medium.

The different parameters discussed in Table 32 shall now be described for the three barley varieties in the N, K, and P series (Fig. 15–17) as related to percentage representations of the 2N2, 2K2, 2P2 level of Sv 59135 (Table 32). The variety Sv 59135 is symbolized by “Sv” and the isogenic six-row and two-row lines are designated “6” and “2”, respectively. Sv is generally superior in tillering capacity and seed yield, and it has a low plant height compared with 6 and 2 in all dressings (Fig. 15). Variety 2 naturally has a higher seed weight than its isogenic sister line 6, which has a higher number of seeds per spike. Seed yield and plant height (Fig. 16) are approximately comparable for these two lines. Line 2 generally has a higher crude-protein percentage in the seed than 6 and Sv except with the 5N2 dressing. Crude-protein yield in seed is superior in Sv as compared with 2 and 6. Ash content is in most dressings highest for Sv, with 6 being slightly superior to 2. In phosphorus, Sv is likewise higher, 2 and 6 giving almost identical values over the whole range of contrasting fertilizer levels (Fig. 17). Potassium follows the same pattern as the ash content with decreasing K percentage from Sv, 6, and 2. Variety 2 is comparatively resistant to high sodium doses when compared with Sv and 6.

In conclusion, several parameters, although strongly affected by environment, also show an influence due to variety that in some cases could be generalized over a whole range of N, P, and K dressings. An example is crude-protein percentage, which certainly is highly variable but which also regularly shows a higher content in the two-row line as compared with its isogenic six-row sister line. The conclusions translated from these artificial conditions over to field trials should be drawn with utmost care. However, the superiority in crude protein of the two-row

Table 32. Analyses of barley variety Sv 59135 grown in perlite and in soil with different nitrogen dressings

Dressing	Number of tillers	Seed weight (mg)	Seed yield (g)	Plant height (cm)	Crude protein in seed (%)	Ash in seed (%)	P in seed (%)	K in seed (%)	Na in seed (%)	Fe in seed (mg/100g)	Lysine (g/16 g N)	Protein yield (mg)	Lysine yield (mg)
<i>Perlite</i>													
5N2	5.0	33	2.8	75	17.5	3.5	0.44	0.85	0.097	3.2	2.58	495	13
2N2	5.4	35	3.1	79	15.9	3.1	0.42	0.80	0.336	4.2	3.45	487	17
2N2	4.9	35	3.5	79	15.5	3.0	0.46	0.80	0.334	3.3	2.93	538	16
Mean* (B + C)	5.2	35	3.3	79	15.7	3.1	0.44	0.80	0.335	3.8	3.19	513	17
N2	4.8	44	4.3	82	8.4	2.4	0.36	0.69	0.139	3.0	4.06	362	15
3N1	3.2	42	2.6	75	6.2	2.2	0.33	0.65	0.047	2.5	4.50	160	7
N1	2.3	38	1.1	74	6.0	2.2	0.32	0.65	0.027	3.0	4.52	63	3
<i>Soil</i>													
2N2	4.7	38	2.5	84	15.3	2.4	0.42	0.68	0.056	3.9	3.34	389	13
N1	3.7	44	3.1	84	9.2	2.4	0.35	0.63	0.024	3.3	3.78	283	11
ON	3.7	45	2.5	79	9.3	2.5	0.35	0.64	0.027	4.2	3.76	236	9

* Standard condition = 100 in Fig. 15—17.

Nutrients (for use, see text):

N1 = 56 g NaNO₃/lN2 = 300 g NaNO₃/lP1 = 20 g NaH₂PO₄/lP2 = 200 g NaH₂PO₄/l

K1 = 50 g KCl/l

K2 = 150 g KCl/l

Solution I: (500 g CaCl₂, 250 g MgCl₂ in 5 l H₂O)Solution II: (50 g Na₂SO₄ in 5 l H₂O)Solution III: (0.100 g CuCl₂, 4.0 g MnCl₂, 6.0 g H₃BO₃, 0.625 g Na₂MoO₄, 0.400 g ZnCl₂, 27 g FeCl₃ + 76 g EDTA in 5 l H₂O)

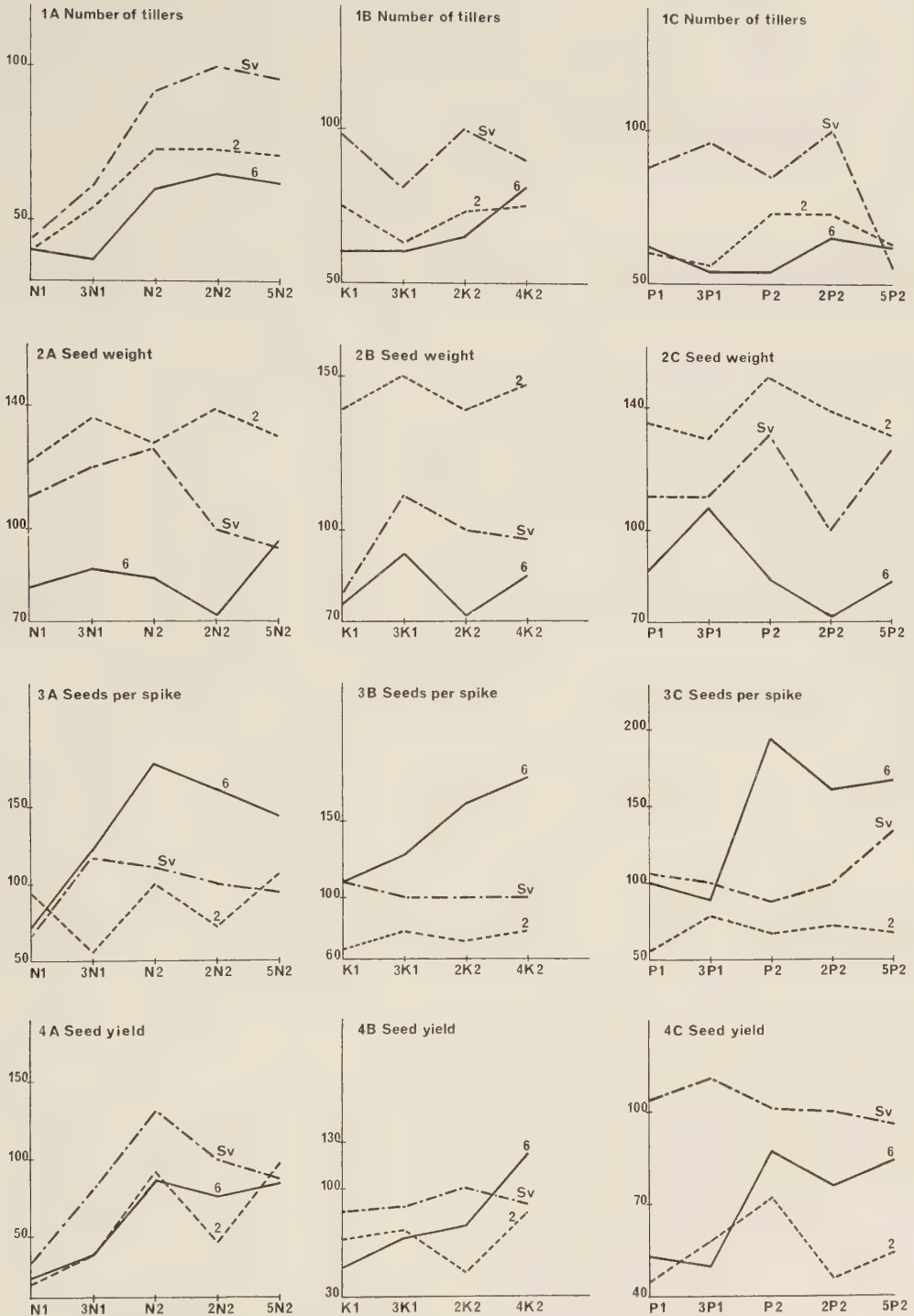


Fig. 15. Fertilizer experiment with the barley variety Sv 59132 (Sv) and with a two-row (2) line isogenic to a six-row (6) line. Nitrogen (N1, 3N1, N2, 2N2, 5N2), potassium (K1, 3K1, 2K2, 4K2) and phosphorus (P1, 3P1, P2, 2P2, 5P2) were applied according to Table 32 and text. Relative figures of number of tillers, seed weight, seeds per spike, and seed yield are given with the 2N2, 2K2, 2P2 fertilizer treatment of Sv 59132 arbitrarily set at 100.

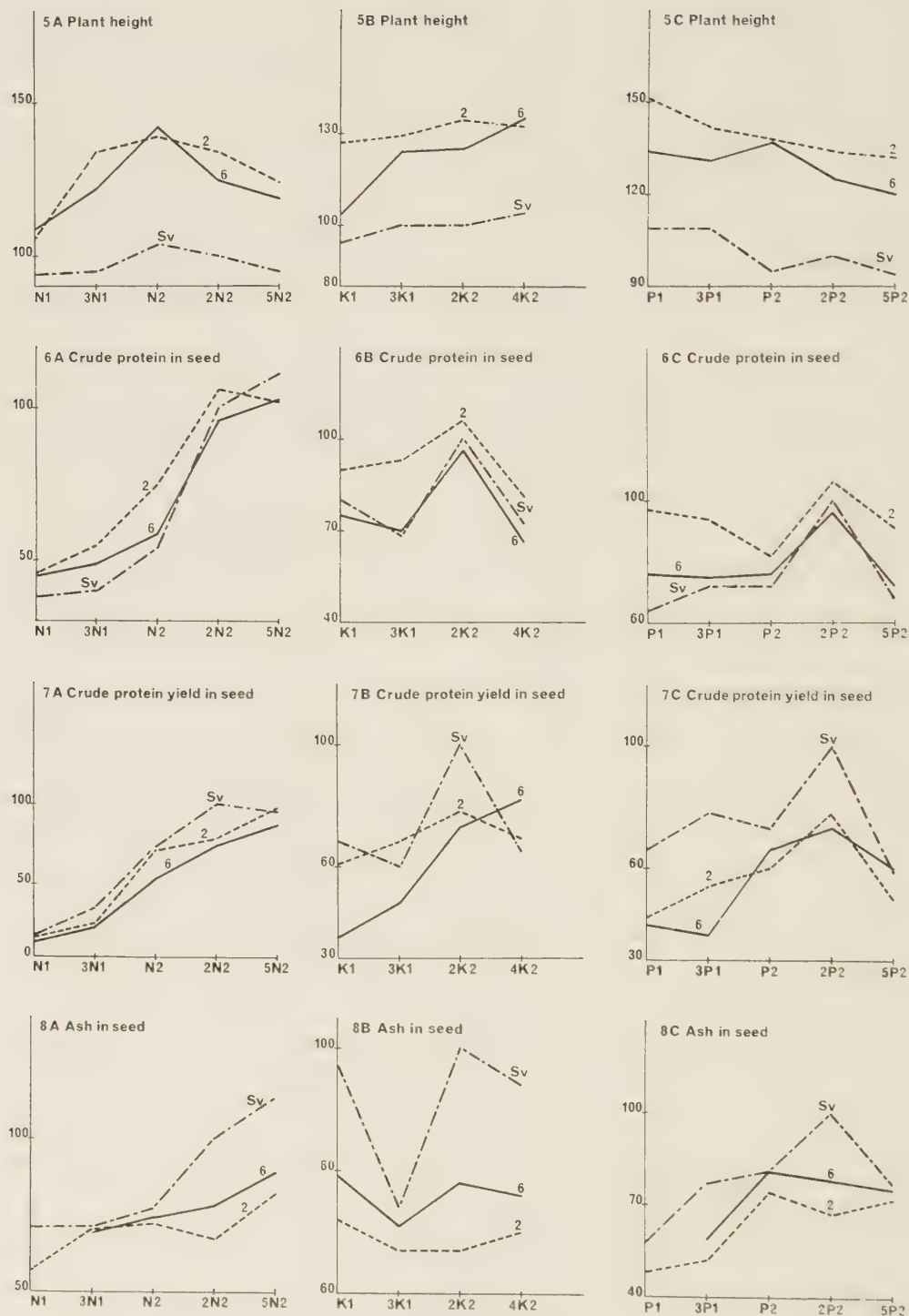


Fig. 16. Fertilizer trial according to Fig. 15. Relative figures of plant height, crude protein yield in seed, and ash in seed are given with the 2N2, 2K2, 2P2 fertilizer treatment of Sv 59132 arbitrarily set at 100.

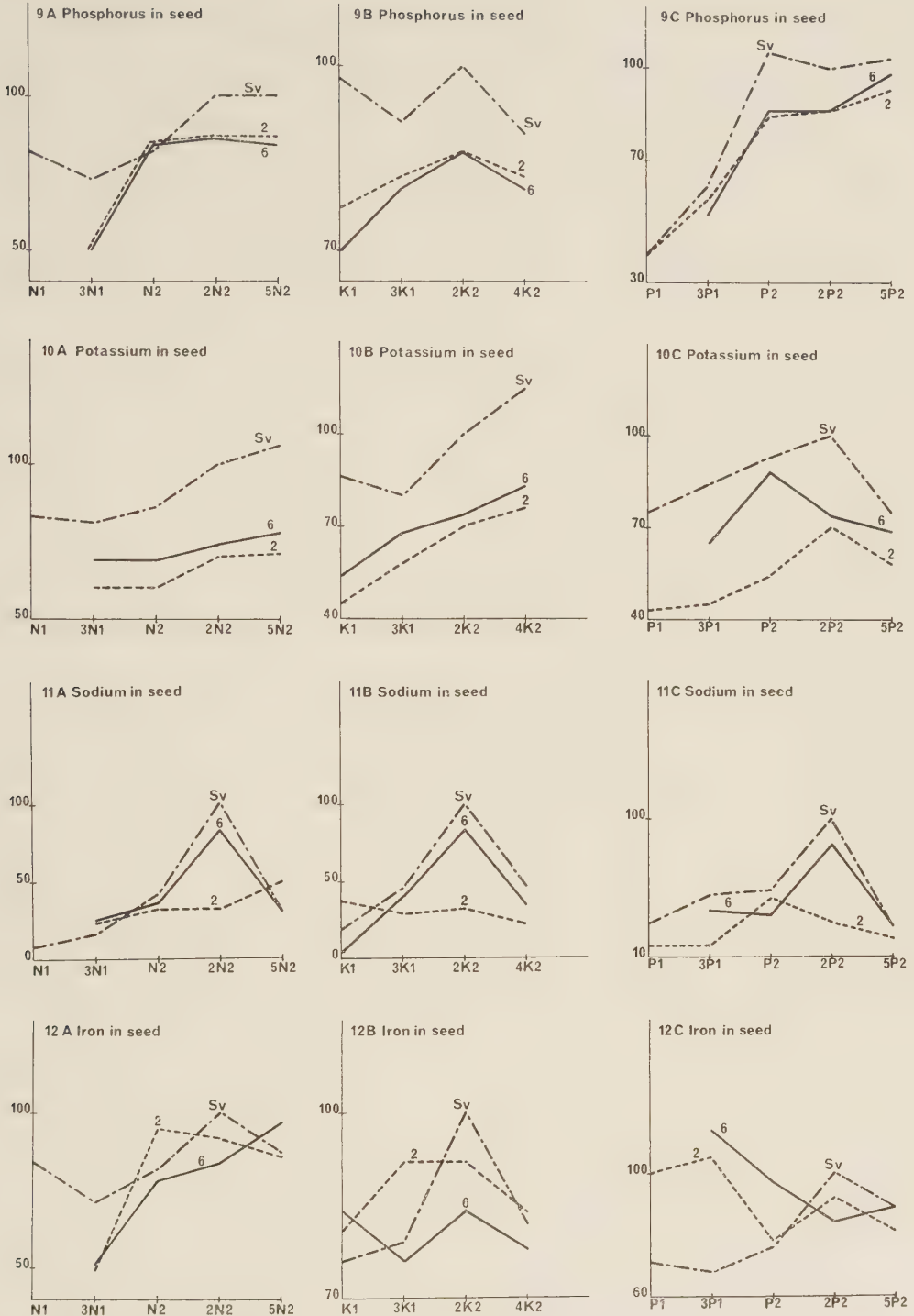


Fig. 17. Fertilizer trial according to Fig. 15. Relative figures for phosphorus, potassium, sodium, and iron in the seed are given with the 2N2, 2K2, 2P2 treatment of Sv 59132 arbitrarily set at 100.

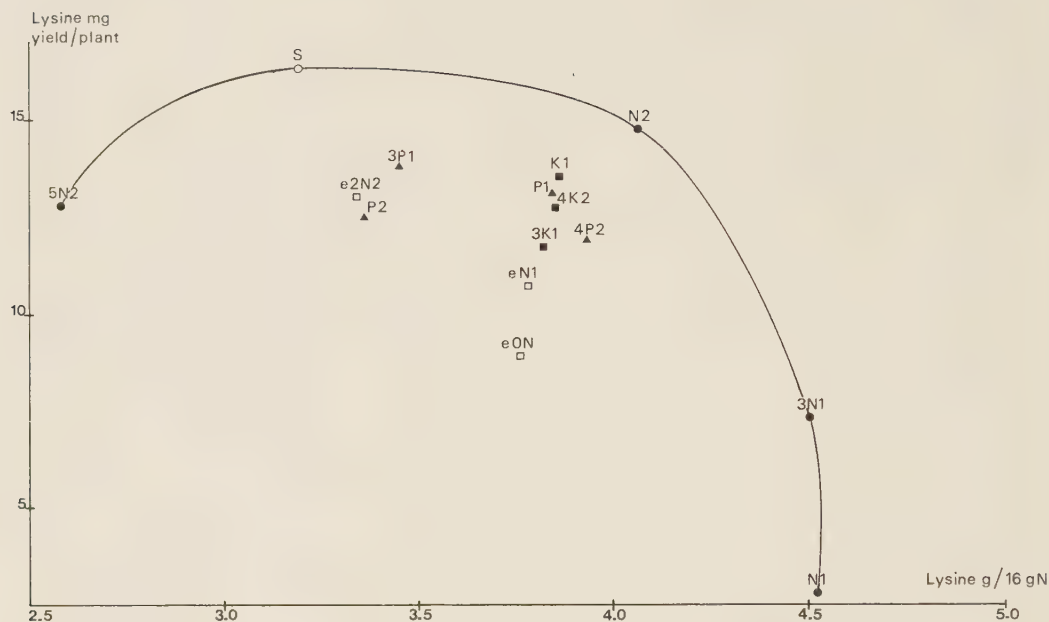


Fig. 18. Fertilizer trial according to Table 32. Lysine yield (mg/plant) as related to lysine in crude protein (g/16 g N) for the barley variety Sv 59132. Standard treatment (S) = 2N2, 2K2, 2P2. Plants were grown either in a soil medium (e) or in perlite.

(20–30 %) over the six-row line has been shown regularly for 10 isogenic pairs during different years in field trials (HAGBERG 1963 and KARLSSON pers. comm.). Because seed size occasionally is negatively correlated with crude-protein and mineral content, the isogenic, six-row lines should be expected to have a higher protein and ash content than their two-row counterparts. The opposite is, however, the case with regard to protein, whereas the expected increase in ash is verified. A six-row mutation material from the two-row varieties Bonus and Foma was obtained from GUSTAFSSON and LUNDQVIST (pers. comm.). Four of the 26 mutant lines were equal and 22 were higher in protein content when compared with their parent varieties. The mutants with the highest protein content displayed a reduced seed size. Thus, the two-row allele is not pleiotropic with respect to an increased protein content in the seeds. HARLAN (1968) points out that either six-row barley was derived from the wild, two-row forms in very ancient times or the progenitor of six-row barley has become extinct. The two-row and six-row cultivated varieties still are two

distinctive groups differing in many characters. Two-row $\times$ six-row crosses, however, occasionally practised, are therefore difficult for the plant breeder to use because of the variety of recombinants with unusual yield characters. Thus, it seems likely that genetic factors controlling the protein content since ancient times are associated with the two-row allele, whereas the six-row barley developed from different progenitor forms carries other genetic factors for protein.

The fertilizer trial described was rather unbalanced even with respect to the standard condition, viz. 2N2, 2K2, 2P2. With additional K and P, protein content (Fig. 16) was markedly reduced and seed weight (Fig. 15) increased in most combinations, reflecting the improved N:K:P balance. Lysine yield mg/plant is negatively correlated with lysine g/16 g N (Fig. 18) for the variety Sv 59135, the only line which was analysed for amino acids. The N series gives the optimal yield performance at the standard dosage. All other combinations with P and K, and with N in soil, give lower yields at similar lysine g/16 g N levels. It could also be concluded that

yields in lysine, crude protein, and in seeds do not coincide in this laboratory trial.

In fertilizer pot experiments comparing opaque-2 maize with normal cultivars (WILBERG et al. 1968), lysine in percentage of protein decreased in normal corn varieties but not in opaque-2. The Osborne protein fractions were also unchanged. Preliminary fertilizer trials with barley (KARLSSON and MUNCK, unpubl.) demonstrated relatively small dressing effects on lysine g/16 g N in Hiproly even when protein varied appreciably.

IV. The use of genetic deviates in cereal chemistry to elucidate how quality-oriented changes arise in seed

1. Introduction to endosperm ultrastructure

Before discussing the biochemical effects of the high-lysine genes in maize and barley, the ultrastructure of the developing cereal endosperm, will briefly be considered.

BUTTROSE (1963) has thoroughly studied the development of wheat endosperm in the electron microscope. The endosperm arises after a triple nuclear fusion. Subsequent free divisions of the nucleus take place without formation of cell walls. Thus, the endosperm sac is lined with free nuclei. Cell-wall formation takes place 2 days after fertilization, after which cell organelles are recognizable. The outer nuclear membrane is extended in sac-like proliferations, and BUTTROSE suggests a connection with the membrane system formed by the endoplasmic reticulum.

Extensions of the inner nuclear membrane were also observed. Protein granules surrounded by a single membrane began appearing about 1 week after fertilization. They were associated with small vesicles resembling the dictyosomes of the Golgi complex.

Starch granules were observed only within well-developed plastids, small starch grains appearing between the large ones and included in a common plastid membrane. This membrane later extrudes and becomes constricted, in turn releas-

ing the small starch granules into the cytoplasm (BUTTROSE 1963).

HESS (1955, cit. ROHRlich et al. 1969), studying the mature wheat endosperm, classified the proteins into two groups that were obtained by density fractionation in liquid medium. The proteins consisted of wedge-shaped deposits separated from the flour and the adhesive proteins, which were attached in the form of fibrils to the surface of the starch granule. BUTTROSE (1963) suggested that the adhesive protein corresponds to a combination of the plastid stroma and the plastid membrane, whereas the "wedge" protein consists mainly of protein granules and cytoplasm.

Discrete protein deposits have been known and recognized in plant cells for more than 100 years (see review by DECHARY and ALTSCHUL 1966). These structures were called aleurone grains, and various kinds were observed in dicotyledonous seed tissues as well as in the central and periferal parts of the monocotyledonous endosperm. In the aleurone cell layer of cereal grains, the "aleurone bodies", are especially conspicuous. It is evident that these aleurone bodies of various kinds, prevalent in seeds, constitute the major storage structures for the so-called storage proteins, which function to furnish amino acids or nitrogen to the germinating seeds. These proteins were called "aleurins" by DECHARY and ALTSCHUL (1966) to distinguish them from cytoplasmic proteins external to the aleurone grains.

In the literature, the terms "protein body" or "protein granule" are generally used instead of aleurone grain. It can be maintained that no general systematic comparative study as regards the protein bodies has been carried out up to now. Thus, different kinds of protein bodies with varying functions and evolutionary history may be present even within the same seed, e.g., in protein bodies from the aleurone layer and in the central endosperm of cereals.

In 1963, MORTON et al. published a series of papers consisting of an interdisciplinary attack on protein synthesis in the wheat endosperm. With a refined electron-microscopic technique, JENNINGS, MORTON, and PALK (1963) demonstrated that the protein body was lined by a lipoprotein membrane and further appressed to a parallel array of lipoprotein membranes, which form a lamellar structure. This system was in close con-

tact with double membranes, which were probably part of the endoplasmic reticulum. The size of the protein bodies of wheat varied from 0.5 to 15 μ .

GRAHAM, MORTON and RAISON (1963) developed a technique for recovering protein bodies from unripe wheat endosperms by using a detergent extraction followed by ultracentrifugation in a density gradient. The proteins from the protein bodies were extracted with acetic acid and analysed by means of starch electrophoresis. They were shown to contain a slow-moving component, which also could be obtained by extracting gliadine from whole-wheat endosperm. Protein fractions in the supernatant were comparable with salt-soluble, fast-moving proteins from whole endosperm. The protein bodies fractionated were studied in the electron microscope, their sizes were mostly about 1 μ and the larger protein bodies accompanying the starch grains in the separation procedure. The protein content ($N \times 6.25$) of the protein bodies isolated was 72 %. By employing a modified ultracentrifugation method, CHRISTIANSSON *et al.* (1969) isolated protein bodies from mature maize endosperm having a Kjeldahl protein content of 86 %. The protein bodies were comprised mainly of the prolamine zein.

KHOO and WOLF (1970) in their study of the origin and development of protein granules in maize endosperm cogently demonstrated that the granules, or bodies, develop from vesicles formed by the endoplasmic reticulum as small cisternal dilations and enlargements of the ends of the endoplasmic reticulum. Dictyosomes from the Golgi apparatus also appeared to proliferate protein granule vesicles. Polyribosomes collected near the outer membranes of the protein bodies. The proteins inside the protein bodies thus seem to be synthesized outside the membranes. The immature bodies stained with a variety of dyes, whereas mature granules stained only lightly, or not at all. At kernel maturity, the protein granules were embedded in a protein matrix (cytoplasmic proteins outside the cell organelles).

Thus, it is evident that genes affecting size and number of the protein bodies may greatly influence the amino-acid composition of the cereal endosperm. Other possibilities in altering the over-all amino-acid composition of the endosperm cell should also be considered, such as a changed composition of the matrix proteins or

of the lipoproteins in the membrane complexes. When changes in protein synthesis would occur, effects on ultrastructure should also be expected, while morphological changes sometimes, but not always, may be interpreted as an indicator of a deviating protein metabolism.

In previous studies (MUNCK 1970a), it was shown that the increase of lysine and other essential amino acids in high-lysine barley was due to a major change in the endosperm. The question is now if the effect is concentrated in a certain part of the endosperm, e.g., in the aleurone layer, which is rich in water-soluble proteins of high nutritional value. Because of the thick cell walls of the aleurone layer, this region of the endosperm is broken down into coarser particles than the inner endosperm when milled in a hammer mill. By sieving it is thus possible to recover a crude fractionation of the outer parts (> 120 mesh) and the inner parts (< 120 mesh) of the endosperm (MUNCK 1964a). In Table 33, the amino-acid composition of the whole-milled seed is compared with the fine and the coarse fractions with regard to Hiproly and the sister line (CI 4362).

It is interesting to observe that both varieties have a high percentage of the coarse fraction (about 60 %). Hiproly is a little lower in this respect compared with the sister line. A large amount of the coarse-meal fraction is thus not generally associated with a hard endosperm (expressed as the starch-protein adherence character) as exemplified by the sister line, which presents free starch grains in meal preparations. The crude-protein content of Hiproly compared with the sister line is 3 % higher in the whole meal and 14 % higher in the fine meal, but 4 % lower in the coarse meal. Hiproly contains 21 % more crude protein in the whole meal in the fine fraction as compared with the sister line.

With regard to the amino-acid pattern of the whole-meal protein, Hiproly is superior to the sister line in lysine (+35 %), histidine (+8 %), arginine (+13 %), aspartic acid (+30 %), threonine (+11 %), glycine (+10 %), alanine (+19 %), valine (+13 %), methionine (+14 %), isoleucine (+8 %) and inferior with respect to NH_3 (-13 %), glutamic acid (-14 %), proline (-16 %), and cysteine (-14 %). If the protein from the fine-meal fractions of the two lines is compared, essentially identical differences are demonstrated, as were observed in the whole

Table 33. Relative amino-acid composition of proteins from whole meal and milling fractions from Hiproly and CI 4362 (= 100)

	Whole seed		Fine fraction < 120 mesh		Coarse fraction > 120 mesh	
	CI 4362 (g/16 g N)	Ratio	CI 4362 (g/16 g N)	Ratio	CI 4362 (g/16 g N)	Ratio
Lys	2.95	135	2.90	134	3.12	125
His	1.93	108	1.94	108	2.03	100
NH ₃	3.21	87	3.23	85	3.21	84
Arg	4.25	113	4.11	115	4.33	99
Asp	4.87	130	4.73	128	5.12	122
Tre	3.08	111	3.08	108	3.22	106
Ser	4.33	100	4.18	103	4.43	96
Glu	25.22	86	25.21	86	25.49	84
Pro	12.80	84	12.84	84	12.85	82
Gly	3.41	110	3.39	107	3.59	103
Ala	3.28	119	3.15	125	3.47	112
Cys*	1.92	86	1.88	82	2.04	79
Val	4.32	113	4.33	112	4.52	109
Met*	1.77	114	1.68	124	1.80	110
Ileu	3.33	108	3.33	105	3.49	102
Leu	6.50	103	6.42	104	6.74	98
Tyr	3.35	97	3.16	101	3.34	99
Phe	5.51	98	5.42	100	5.65	95
Sum (mmol/kg)	1559	97	1476	112	1647	91
Crude protein (%)	17.20	103	16.60	114	17.80	96
Fraction (% dry matter)	100	100	37.5	105	62.5	97
Fraction (% crude protein)	100	100	36.2	121	64.5	94

* Oxidation procedure

meals. Methionine is, however, here 24 % higher in Hiproly compared with 14 % in the whole-meal protein. On the other hand, the coarse fraction shows less difference between Hiproly and the sister line for most amino acids, and especially lysine, histidine, arginine, aspartic acid, glycine, alanine, and methionine. If we regard Hiproly and the sister line as being almost isogenic, the conclusion is that the lys gene affects the amino-acid composition both in the outer and the inner parts of the endosperm, with a slight overweight for the latter.

To obtain more specific data with regard to the morphological effects of the high-lysine trait in Hiproly barley, about 1 μ -thick transverse sections were made with an ultramicrotome. Endosperms were previously fixed in glutaraldehyde, osmium vapor and chromic acid and embedded in Epon. The sections were dyed with toluidine blue.

A low-protein (about 10 %) commercial malting barley, variety Kristina (Fig. 19), is compared

with the Hiproly sister line, CI 4362 (Fig. 20) and Hiproly (Fig. 21). Kristina has much more starch in the large endosperm cells, which extend to the aleurone cell layer. Both the sister line and Hiproly have a well-defined subaleurone layer consisting of cells that are abundant in protein and almost completely devoid of starch granules. This condition resembles that of the hard wheats (KENT 1970). Kristina is thus more comparable to a soft wheat which lacks the subaleurone layer. Kristina also displays a low content of coarse meal, whereas both Hiproly and the sister line exhibit the opposite character. Thus, it is likely that the milling properties are bound more to a thick subaleurone layer than to the starch-protein adherence trait. Hiproly has more irregularly, strongly compressed endosperm cells in the subaleurone layer than the sister line, this fact presumably being responsible for the shrivelled seeds of Hiproly.

A close-up of the subaleurone layer (Fig. 22) and the endosperm (Fig. 23) region reveals a

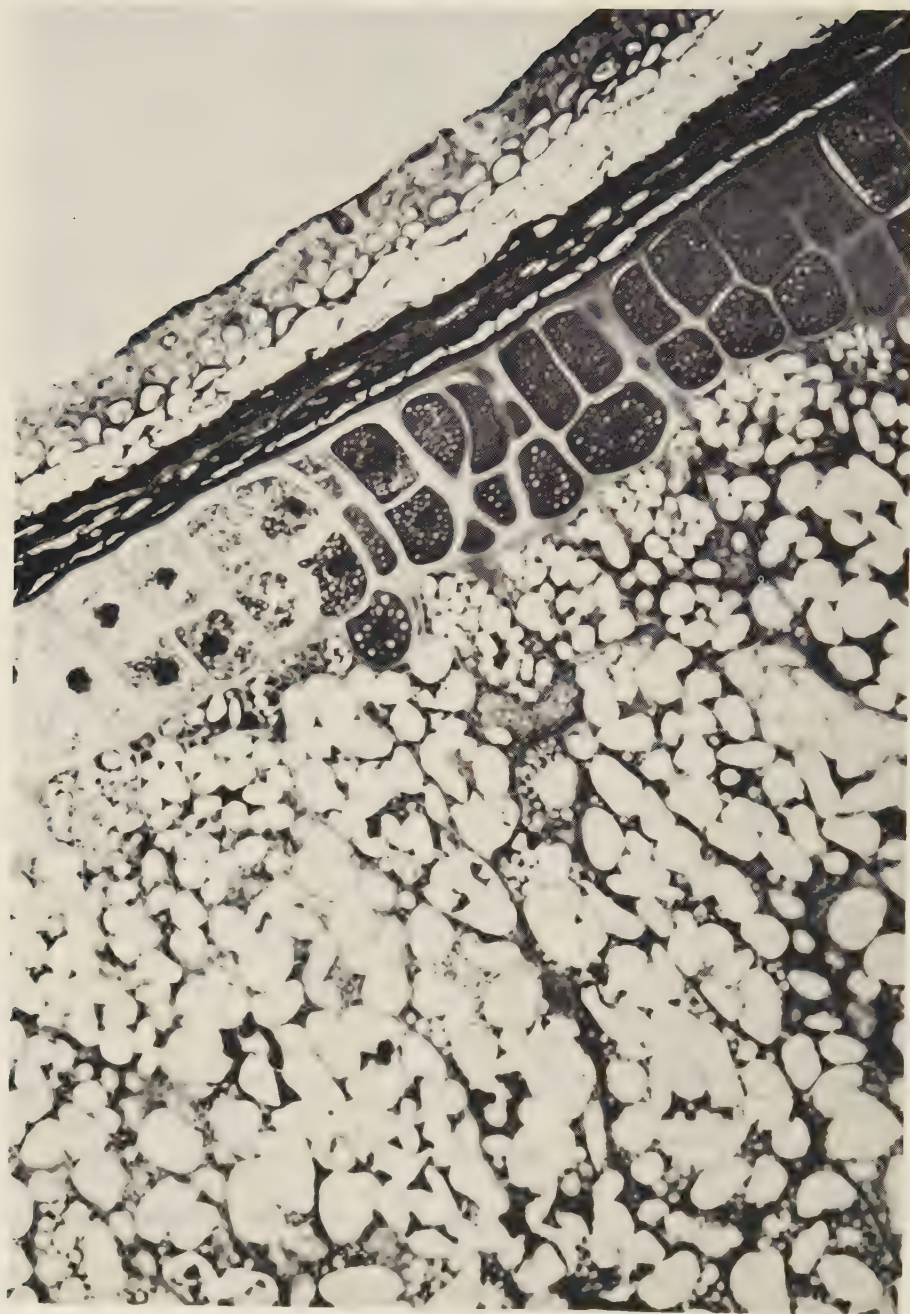


Fig. 19. Endosperm of the barley cultivar Kristina. Transverse section ($1\ \mu$). $\times 400$. Preparation from a dry, mature seed.



Fig. 20. Endosperm of Hiproly sister line (CI 4362). Transverse section ($1\ \mu$). $\times 400$. Preparation from a dry, mature seed.



Fig. 21. Endosperm from Hiproly barley. Transverse section ($1\ \mu$). $\times 400$. Preparation from a dry, mature seed.

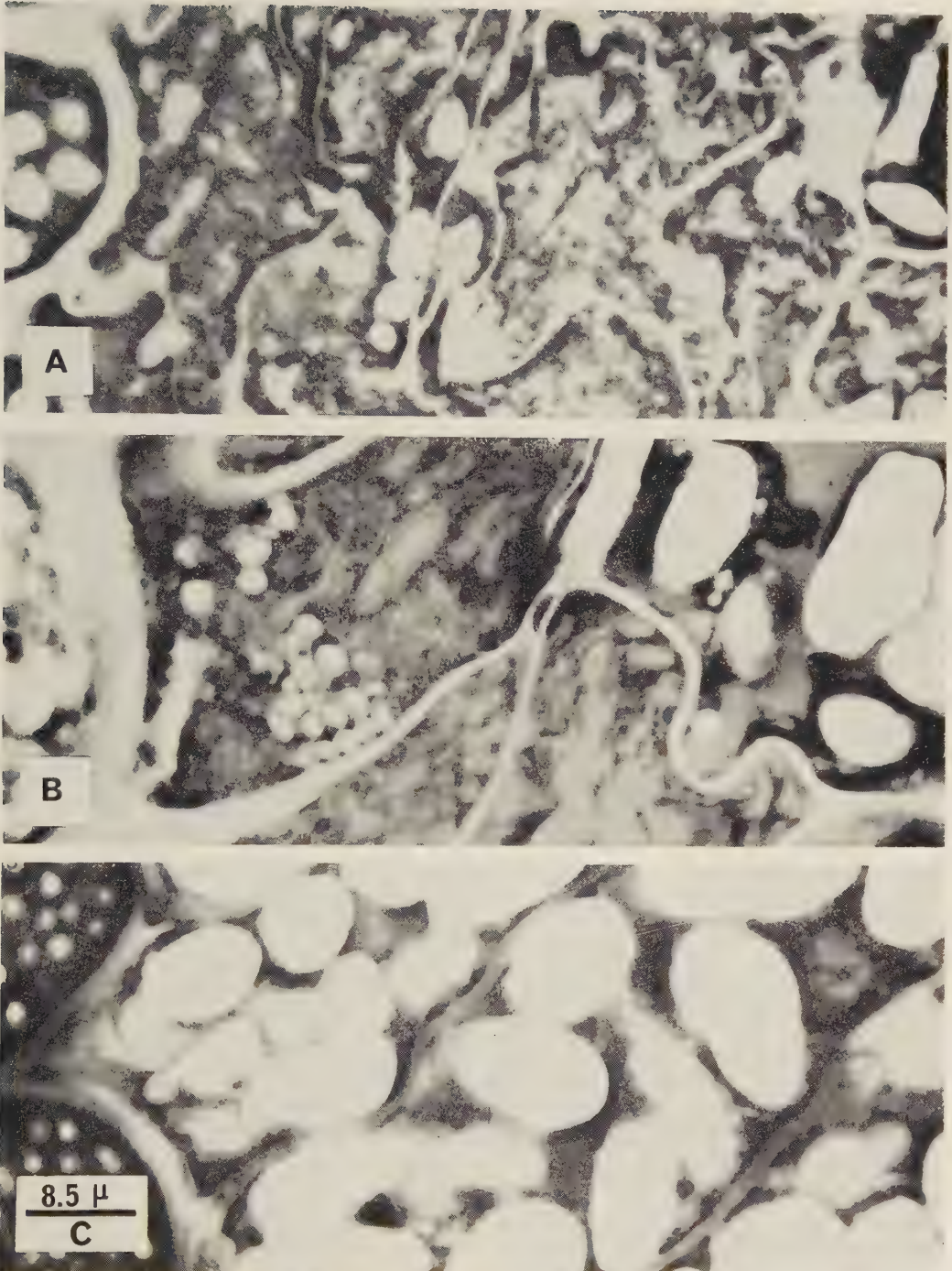


Fig. 22. Subaleurone cells in endosperms from Hiproly (A), CI 4362 (B) and Kristina (C) barleys. Aleurone cells to the extreme left. Preparations from dried, mature seeds.

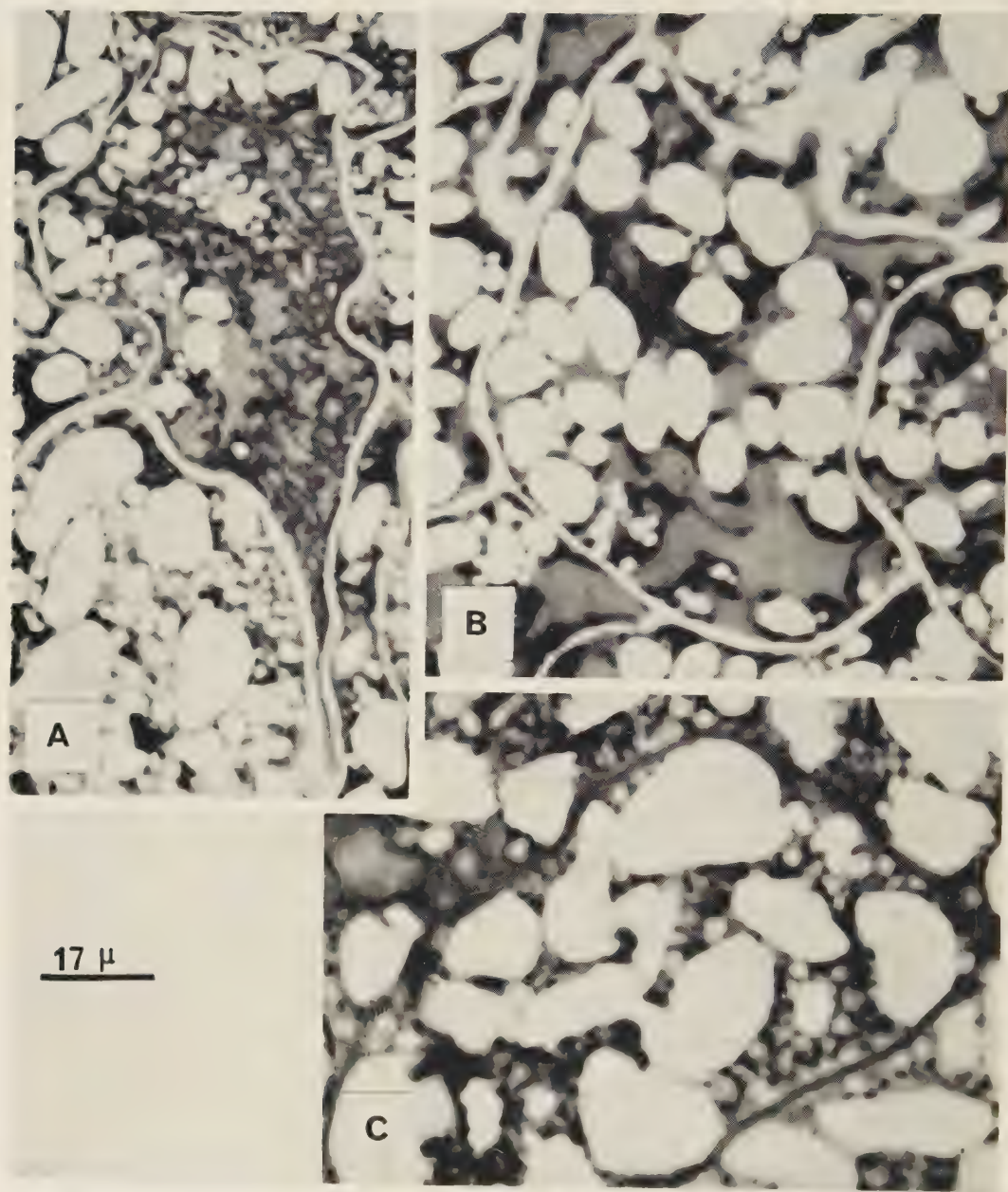


Fig. 23. Central endosperm cells from Hiproly (A), CI 4362 (B) and Kristina (C) barleys. Preparations from dried, mature seeds.

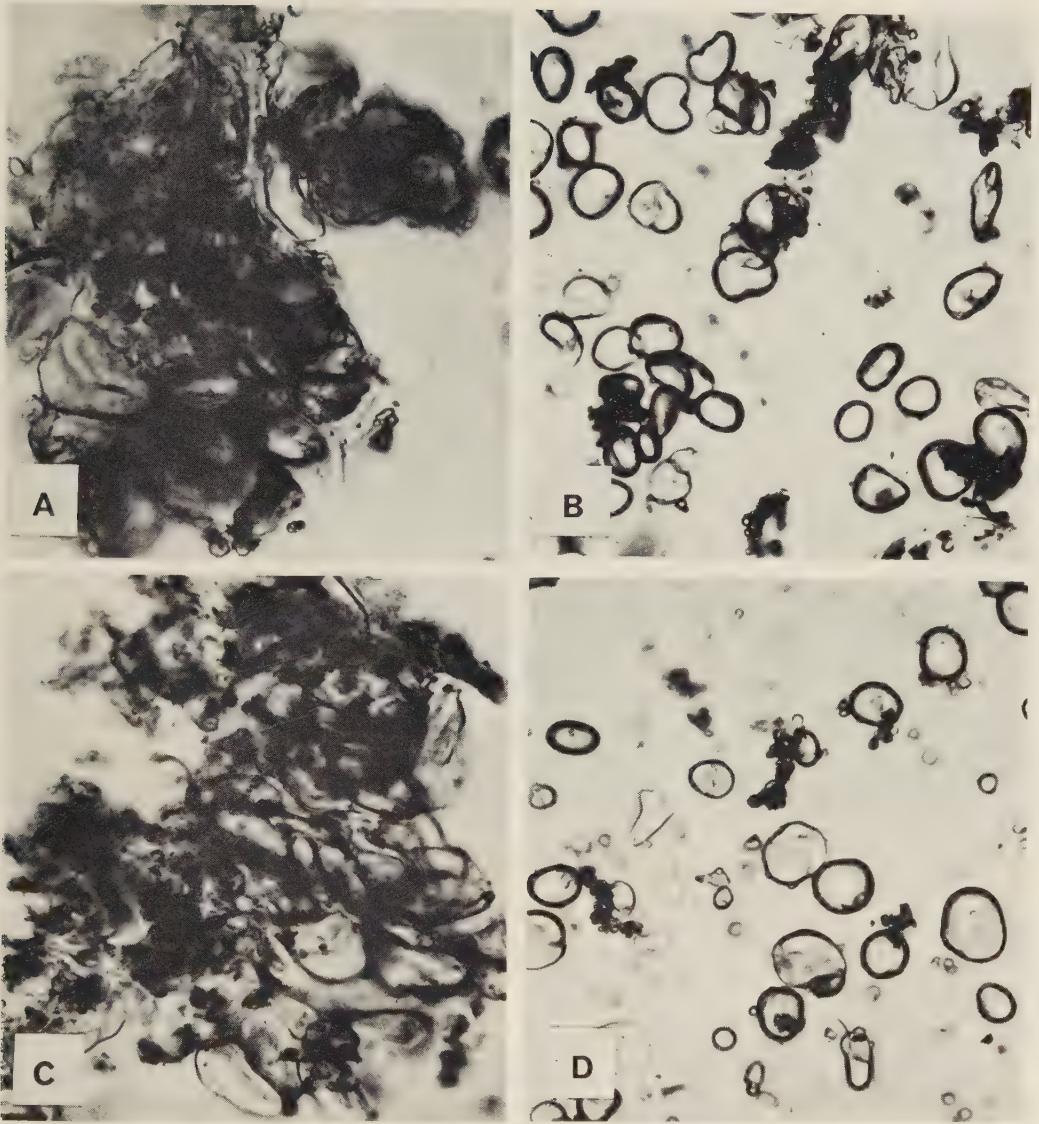


Fig. 24. Light microscopy micrographs from meal preparations displaying the starch-protein adherence character. Preparations from dried ripe seeds of Hiproly (A), normal-lysine barley (B), high-lysine (DBC) segregant C and normal-lysine (DBC) segregant (D). Acilane-Orange dye pH 2.6.

more compressed proteinaceous structure in the subaleurone layer of Hiproly (22:A), with the cell walls almost invisible when compared with the sister line (22:B). In Kristina the cells are packed with starch granules (22:C) and the cell walls are more darkly stained than in the two other lines. In the endosperm, Hiproly displays

a characteristic filamentous protein pattern, especially visible in cells that are devoid of starch grains (23:A). In the other lines (23:B, C), starch granules are much more uniformly distributed in the cells. The sister line lacks the densely stained filamentous pattern typical for Hiproly, but has, on the other hand, larger, more sparsely occur-

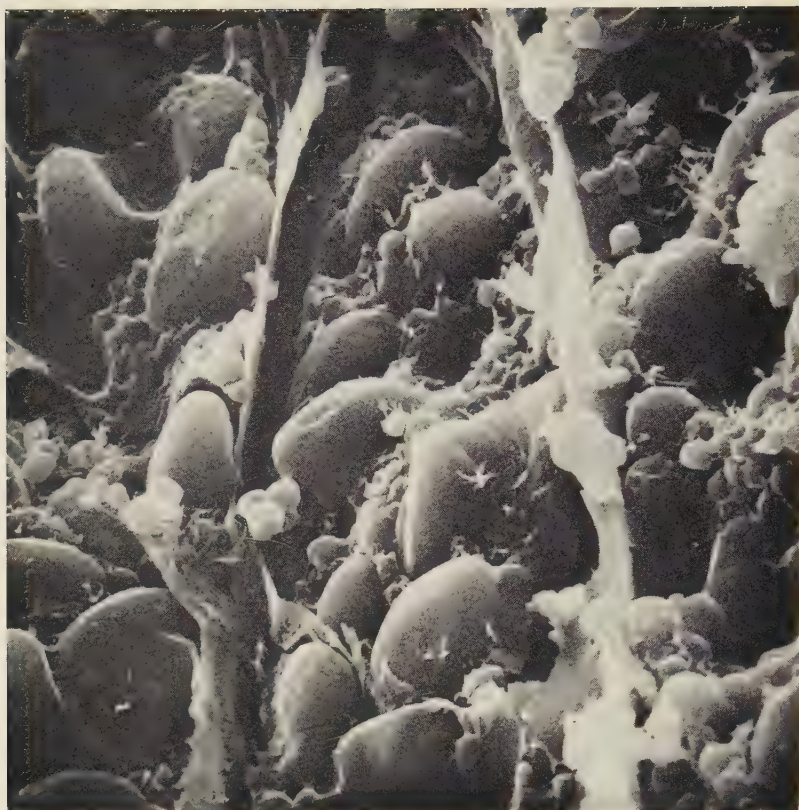


Fig. 25. Scanning electron micrograph from the central endosperm of high lysine barley (Hipoly). $\times 1400$. Preparation from a dry, ripe seed.

ring dark-stained areas in the endosperm cells (23:B). In the low-protein Kristina, filamentous structures resembling those of Hipoly can be seen between the starch grains. In this respect, Hipoly seems more closely related to Kristina than to the sister line. Preliminary studies, not presented here, have shown that the segregants in the F_4 material display a large variation with regard to morphology (e.g. extension of the subaleurone layer) both in the normal and in the high-lysine group, thus opening up new problems for genetic analysis.

With regard to seed texture the seed is more difficult to study in barley than in maize owing to the outer seed coats. The scratch technique was employed to study seed hardness (MUNCK et al. 1970). The kernel is divided transversely with a razor blade and the cut surface is uniformly scratched with a dissecting needle and

then the scrapings are transferred to a microslide; a drop of Acilane Orange G solution (pH 2.6) is added and the cover glass is affixed.

In Fig. 24, Hipoly (24:A) is compared with a normal line (24:B) and with high-lysine (24:C) and normal (24:D) segregants. The characteristic starch protein-adherence picture is clearly seen in the high-lysine lines compared with the normal lines, which exhibit a good starch-protein separation.

These differences are more easily interpreted in the photographs made with the scanning electron microscope (SEM). In Fig. 25 showing Hipoly, the starch grains are evidently firmly attached to the matrix protein. Cell walls, protein bodies, and small starch granules are also seen. A similar view of the normal sister line reveals lucidly that several starch grains and granules have fallen off of the matrix proteins

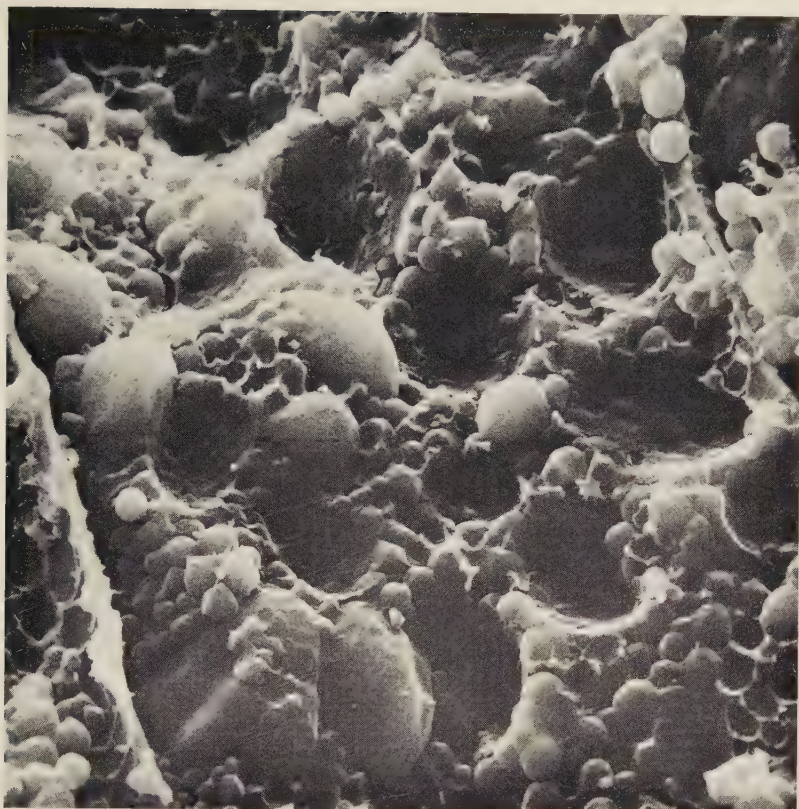


Fig. 26. Scanning electron micrograph from the central endosperm of normal-lysine barley (CI 4362). $\times 1400$. Preparation from a dry, ripe seed.

leaving deep holes (Fig. 26). The surfaces of the cut plane is smoother here than in Hiproly. The starch protein-adherence attribute of Hiproly (24:A) in meal preparations is thus compatible with the SEM picture (Fig. 25) and, likewise, the normal condition (24:B) in the light microscope corresponds to the SEM picture (Fig. 26) of the sister line. From the studies with the two techniques, it is evident that the matrix proteins adhere firmly to the starch grains in the high-lysine lines considered.

The scratch technique also followed the high-lysine (high DBC) trait reasonably well in the study of segregating lines in the F_4 (Table 23). However, in backcrosses, to morphologically normal lysine lines, a wide range of intermediates occurred with semihard, and semisoft endosperms. Therefore, it is difficult to use this character in the screening technique for the lys

character, particularly since morphologically normal, high-lysine recombinants may segregate (Table 23) and because hard morphological lines that are normal in lysine can be recovered, e.g., the variety Monte Cristo. Nevertheless, it is important to study this character to determine whether it is correlated with seed size, or if it has some bearing on the nutritional utilization value of barley (Chapter V).

As was previously described, the protein bodies occur in the endosperm as storage organs for the prolamines. In maize, protein bodies are 1–2 μ in diameter (WOLF et al. 1969), but in wheat larger bodies measuring up to 7 μ were found (BUTTROSE 1963). Protein bodies could be seen in the light microscope in preparations from dry seeds in maize and rice, but this was not possible in wheat owing to their integration with the matrix proteins (WOLF and KHOO 1970). In pre-

parations from frozen seeds from the yellow-ripening stage, no differences could be detected between Hiproly and the sister line regarding the size of the protein bodies. This was verified in SEM studies. Small starch granules and protein bodies could be difficult to discriminate in SEM preparations. Protein bodies should, however, dissolve after a brief treatment (30 sec.) in hot (70°C) ethyl alcohol. In Fig. 27, the central endosperm of CI 4362 at the yellow-ripening stage is shown without and with alcohol treatment (27:A and B, resp.). The seeds were air-dried after being cut with a razor. Large and small starch grains with smooth contours are present in both 27:A and 27:B. In 27:A, small protein bodies, 0.2–3 μ in diameter, can be seen, some of which are irregularly shaped owing to the attached matrix proteins. Most of these protein bodies were dissolved by the alcohol treatment (27:B).

In order to study the effects of different solvents on morphological structures in the SEM, the endosperm (Fig. 28) and subaleurone layer (Fig. 29) of Hiproly (B) and the sister line (A) at yellow-ripening stage were treated with phosphate buffer at pH 7.0 (D, C) and with alcohol (70 %) at room temperature for 5 minutes (F, E). With regard to the central endosperm, a photomicrograph of a cell—including cell wall, starch grains and protein bodies—is seen in Hiproly (28:B), which does not differ particularly from the sister line (28:A). The buffer treatment of the latter variety does not change the picture (28:C), whereas in Hiproly all the large starch grains and most of the protein bodies have eroded away. A few starch grains and protein bodies that are embedded in the matrix proteins are observable. An analogous effect is demonstrated with a cold alcohol treatment, the sister line being almost unaffected (28:E) in comparison with Hiproly, which exhibits large areas of matrix starch and protein bodies (28:F). In the subaleurone layer (Fig. 29), the matrix proteins leach out along the cut surface and cover the starch grains and protein bodies in Hiproly (29:B) and the sister line (29:A). A buffer treatment only incompletely dissolves the matrix protein in the sister line, while this treatment reveals starch grains and protein bodies fully in Hiproly (29:D). In the alcohol treatment (29:F), protein bodies are completely removed in Hiproly compared with the sister line (29:E).

Although these investigations are preliminary and have been run with a limited sample material, it may be concluded that the matrix proteins are more readily removed by solvents from the endosperm and the subaleurone layer in Hiproly than in the sister line. The micrographs shown in Fig. 28 and 29 are representative for the entire seed region in question.

From the studies previously reviewed (WOLF and KHOO 1970) considering the weak-staining properties of mature endosperm compared with non-dried endosperm, we may conclude that the chemical properties of proteins from this tissue are radically changed after drying. To redissolve the proteins involved without excessive denaturation is an extremely intricate problem that was discussed already by OSBORNE (1924). Osborne extraction must be regarded as a very crude but convenient method of fractionation, giving no sharp boundaries between the different classes of proteins. This method was used by BISHOP (1928, 1930) in barley to predict the content of noxious protein for the brewing industry.

2. Protein analysis and synthesis

In Sweden during the 1930's and 1940's, the invention of the ultracentrifuge by SWEDBERG and the moving-boundary electrophoresis by TISELIUS inspired the investigations by QUENSEL, DANIELSSON and SÄVERBORN in one of the most easily extracted protein fractions—the globulins. QUENSEL (1942) determined four components in the globulin fraction (alpha, beta, gamma, and sigma) and SÄVERBORN et al. (1944) found that gamma was present only in the embryo and alpha and beta only in the endosperm.

DJURTOFT (1961) concluded the classical period of extraction analysis in his extensive study of the salt-soluble proteins of commercial barley samples. He concentrated on obtaining reproducible extraction conditions, attempting to depress proteolytic activity by inhibitors (KBrO_3) and avoiding precipitation effects of phytic acid.

Another path of investigation is presented by NIELSEN et al. (1970), who studied glutelin protein of mature maize. After extraction with salt and alcohol solutions, starch was removed with 90 % dimethyl-sulphoxide or digested with alpha

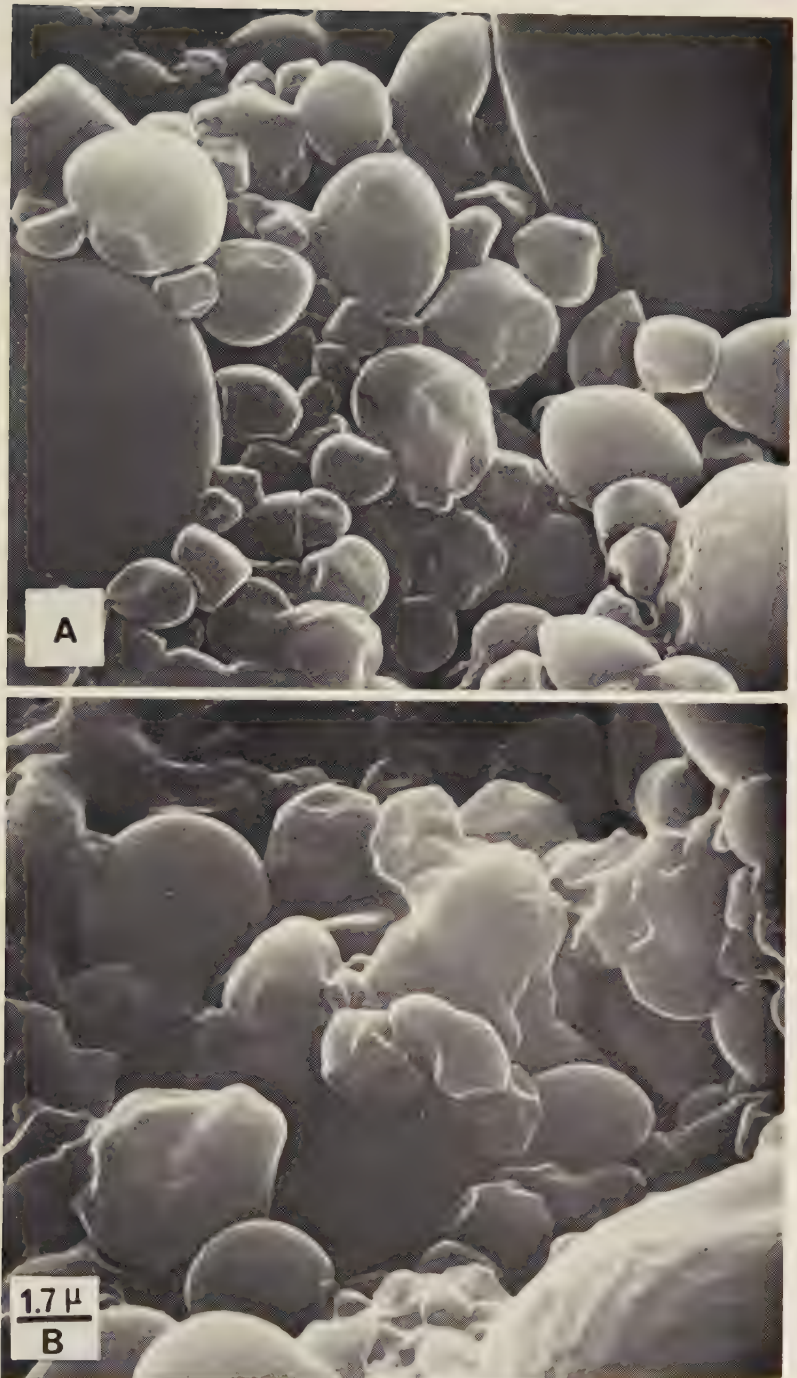


Fig. 27. The effect of treatment of the central endosperm of CI 4362 barley with boiling ethanol (B) as shown in the scanning electron microscope. Untreated control (A). $\times 6000$. Frozen endosperm cuttings from yellow-ripening stage.



Fig. 28. Treatments of barley endosperms (centre) from normal lysine CI 4362 (A, C, E) and high-lysine Hiproly (B, D, F) with phosphate buffer (pH 7.0, 0.1 M; C, D) and ethanol (70 %, 20°C; E, F). Untreated controls A, B. Frozen seeds from yellow-ripening stage. Scanning electron micrographs.

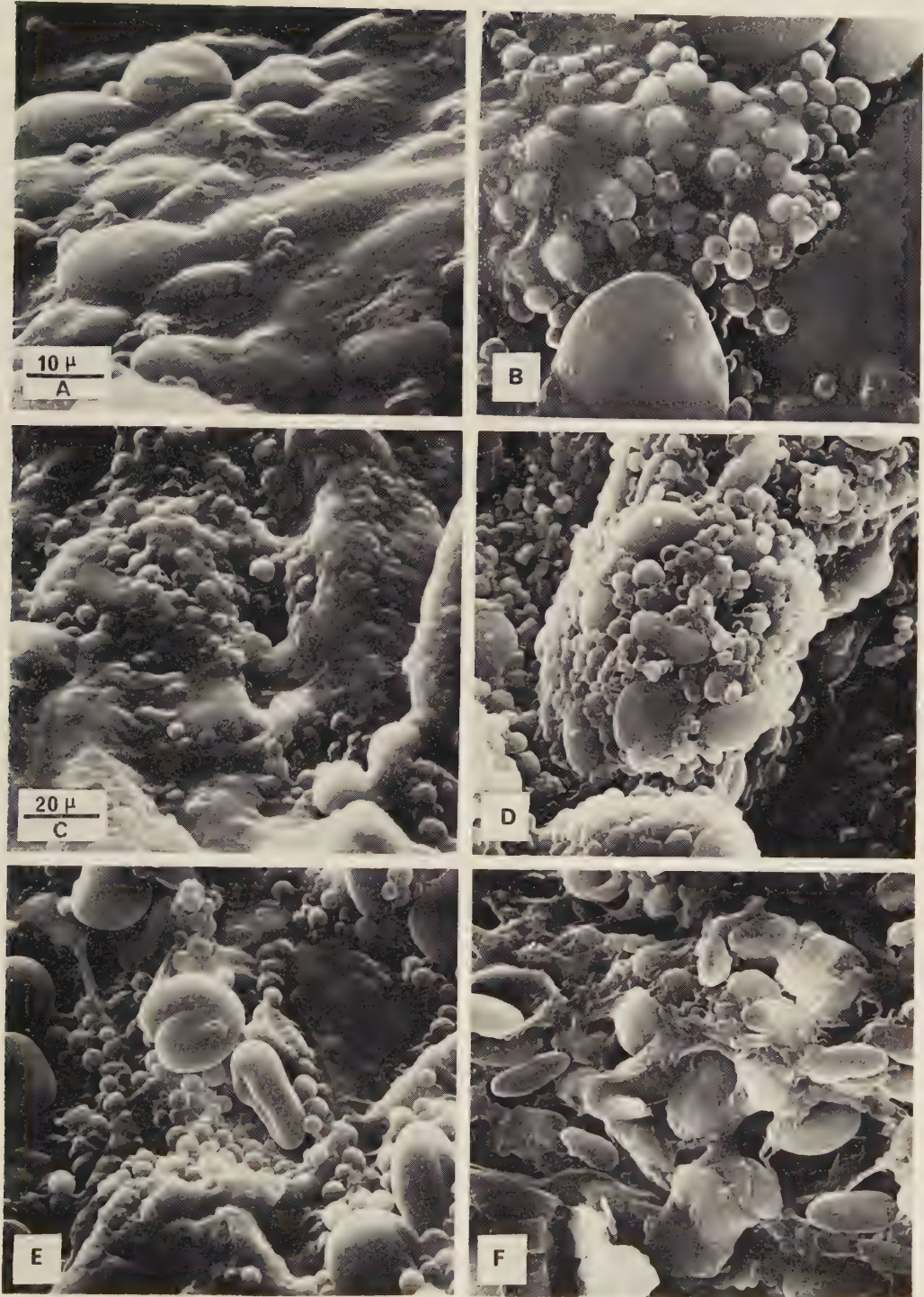


Fig. 29. Treatments of barley subaleurone layer from normal lysine, CI 4362 (A, C, E) and high-lysine Hiproly (B, D, F) with phosphate buffer (pH 7.0, 0.1 M; C, D) and ethanol (70 %, 20°C; E, F). Untreated controls A, B. Frozen seeds from yellow-ripening stage. Scanning electron micrographs.

amylase. A residue insoluble in the most potent solvent was produced from either of the two fractions. The residue became soluble by 2-mercaptoethanol and subsequent alkylation by acrylonitrile when S-S bridges were cleared by reduction. Electrophoretic analyses of modified gluten indicate that the crude-protein fraction is contaminated by zein and other proteins which display a similar migration length in electrophoresis as salt-soluble proteins. On the other hand, it is questionable if such heavy treatments do not cause artifacts, which are difficult to interpret in the native state.

The extraction problem with regard to the cereal proteins is thus very difficult to solve, at least to the satisfaction of the pure chemist. In Fig. 30, the amino-acid percentages in the crude Osborne fraction (MUNCK et al. 1971) are presented for high- and low-protein; normal barley lines and Hiproly are compared. The prolamines in the ethanol-soluble fraction are markedly lower in lysine, aspartic acid, cysteine, and methionine compared with the water- and salt-soluble albumins and globulins. The sodium-hydroxide-soluble fraction is intermediate, whereas the undissolvable protein residue resembles the water fraction more in amino-acid composition. The low-protein normal variety and Hiproly are higher in water-soluble and lower in ethanol-soluble proteins when compared with the high-protein normal line. Hiproly has an increased level of sodium hydroxide-soluble protein than the two other lines. Lysine in Hiproly when compared with the high-protein barley cultivar is consequently higher in the water, salt (potassium sulphate), sodium hydroxide, and residual fractions in contrast to the ethanol and acetic-acid fractions. Hiproly is comparable to the low-protein selection in this respect. *The most striking condition is, however, that cysteine is lower in Hiproly in all the fractions analysed.* Because of the sodium hydroxide treatment, this amino acid could not be recorded in all fractions. *On the other hand, methionine is higher in Hiproly in all protein fractions except the ethanol-soluble one.* The differences as regards sulphur amino acids are further verified in Table 33 employing amino-acid analyses after oxidation – analysing methionine as sulphone and cysteine as cysteic acid. This method is more reliable for obtaining correct absolute values than the method of hydrolysis omitting oxidation used in Fig. 30. Nu-

merous analyses, however, verify that relative figures from non-oxidized analyses are approximately correct.

In a preliminary study of protein synthesis endosperms (3 g) of Hiproly and the sister line (CI 4362), from different developmental stages, were disintegrated in an Ultra Turrax (at 4°C) and extracted twice with water followed by sodium chloride (1.2 %) and ethyl alcohol (70 %). Centrifugation was done in a high-speed centrifuge at 4°C, including two washings. Absolute ethyl alcohol was added to the water extract to make 70 %, and the resulting protein precipitate was obtained by centrifugation. All solutions were analysed by duplicate micro-Kjeldahl analyses and expressed as $\text{mg} \times 6.25/\text{seed}$ (Fig. 31). Crude protein of the insoluble residue per seed was determined as well as total protein content per seed, the latter being obtained by adding the values from the different fractions.

The developing Hiproly endosperms are consistently smaller than those of CI 4362. Per seed, CI 4362 is higher in the amount of crude protein throughout all stages of ripening (Fig. 31). Crude protein increases most rapidly during the short period between July 19 and 28.

The protein content per seed of the ethanol-soluble water extract and the sodium chloride extract is almost constant throughout the entire developmental period, the sister line (CI 4362) being slightly higher with regard to the sodium chloride extract. The amount of the ethanol-precipitable water extract per seed shows, however, a higher level in Hiproly compared with CI 4362 at all stages. This fraction decreases in both varieties from July 13 to July 16, is fairly constant between July 16 to July 23 but displays a distinct increase in Hiproly between July 23 and 28. In the period between July 28 and August 4, the ethanol-precipitable water extract per seed is decreased, presumably owing to the effects of denaturation, which will be discussed below. The values at the July 13 stages may have been affected by the difficulty in dissecting out the embryo and pericarp from the endosperm in these minute samples. This separation was, however, clear-cut and easy to perform at all the other stages. It can also be seen in Fig. 31 that the increase in the ethanol-precipitable protein from the water extract of Hiproly coincides with the most rapid increase in protein from the ethanol extract. CI 4362 is superior with regard to the ethanol crude-protein fraction per seed at all stages. Synthesis was terminated in both varieties on July 28. The insoluble residue

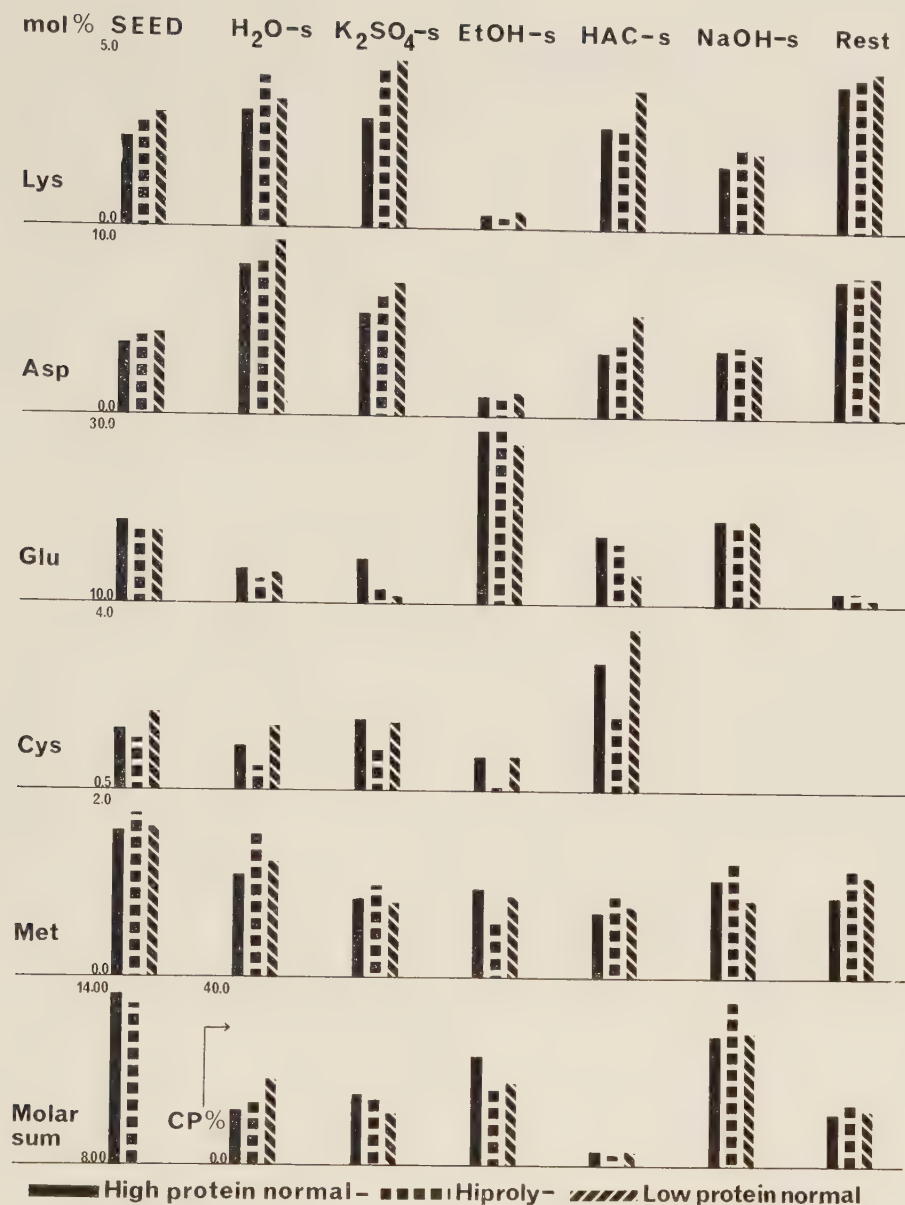


Fig. 30. Amino-acid composition (mol %) of seeds from normal- and high-lysine barleys and of corresponding freeze-dried Osborne protein fractions obtained by successive extractions with water, 5 % K₂SO₄, 75 % ethanol (EtOH), 0.1 M acetic-acid (HAc), 0.5 M NaOH; absolute ethanol 1:1 and residue (rest) fraction. Crude-protein (CP) content of the different fractions is given as a percentage of the crude-protein content of the seed.

Figure

———— Hiproly (673)

----- CI 4362 (675)

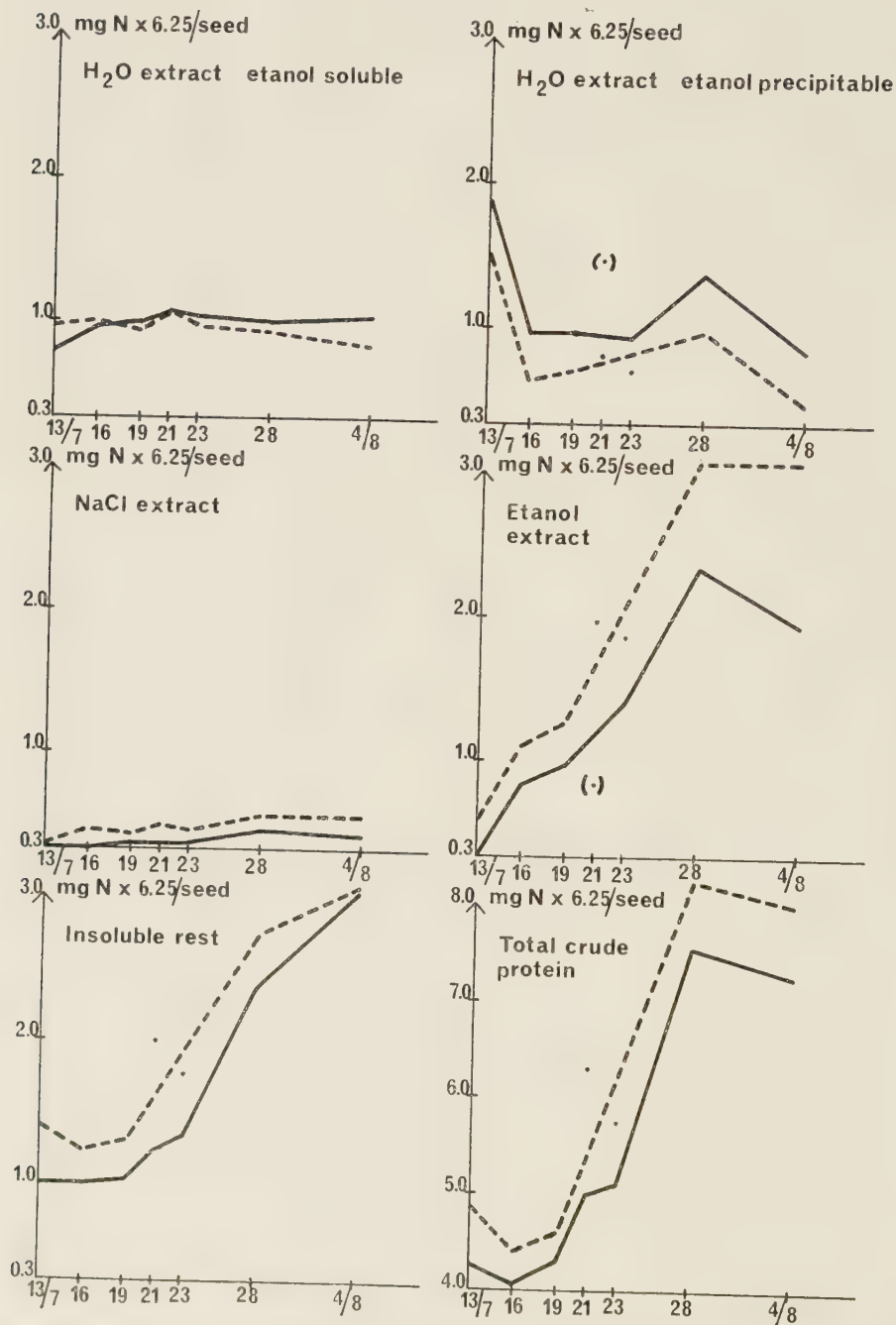


Fig. 31. Protein fractions from high-lysine (Hiproly) and normal barley (CI 4362) endosperms during development. Values are expressed in mg N x 6.25 per endosperm as related to date. Heading time for Hiproly June 23 and for CI 4362 June 22.

per seed shows a similar growth curve, with CI 4362 being higher than Hiproly, and these differences were reduced at the late stage on August 4 (yellow-ripening stage seeds containing about 40 % water).

It is evident that the relative differences on a seed basis between Hiproly and CI 4362 will be enhanced on a crude-protein basis with respect to the ethanol-precipitable water extract, owing to the higher content of total crude protein per seed in CI 4362 as compared with Hiproly. Similarly, the varietal differences in the ethanol extract will be reduced, calculated on a total crude-protein basis.

To obtain a more specific understanding of the protein synthesis, polyacrylamide electrophoretic separations (MIKOLA 1965) were performed (Fig. 31–34). Water and salt (0.2 M barbital pH 8.6) extracts were made consecutively on single endosperms using 200 μ l and 100 μ l, respectively. Homogenization was performed with a glass pestle and the suspension was allowed to stand overnight at 4°C, after which centrifugation was carried out, followed by a single water wash. Electrophoretic separations were run in 15 % polyacrylamide gel to the anode and cathode in 0.1 M tris buffer at pH 8.6. Formic acid (0.1M)-soluble proteins, extracted after the water and salt fractions, were run at pH 5.0 in a beta-alanine buffer towards the cathode in 7.5 % gels. The gels are charged directly with 10 μ l extract from a constriction pipette omitting the sample gel. The gels were coloured with Coomassie Brilliant Blue R-250 (1 % solution diluted 35 times in 10 % TCA) and decolourized 24 hours in TCA. Varieties with the same treatments were usually run together, migration length being not fully reproducible in different runs.

In Fig. 32, electrophoresis at three sampling times with endosperms at water contents of about 65 %, 40 % and 10 % (mature dried seed) are compared for Hiproly, CI 4362 and Kristina. The first two water content figures correspond to the approximate developmental stages, early milk stage (July 16–21, Fig. 31) and yellow-ripening stage (July 28–August 4). It is seen in Fig. 32 that Hiproly manifests much more intense bands at 40 % water content in the anodic separation of the water-soluble proteins when compared with Kristina and CI 4362. It is also obvious, comparing the 65 % water stage, that Hiproly starts earlier than the other varieties to synthesize a specific strong band with a medium migration velocity at the anodic separations. At this stage, several bands are visible that seem to have no counterparts at the 40 % moisture stage. Several of the bands at the latter stage therefore

seem to be newly synthesized and should be included in the peak obtained in the ethanol-precipitable water fraction on about June 28 (Fig. 31). Some of the anodic bands extracted by salt and run at pH 8.6 are also found in the water-soluble fraction, varietal differences in this extraction being small.

In the cathodic separations (Fig. 32) of water and salt extracts, no distinct bands are seen in the early stage (65 % water). At the later stages, a diffuse band pattern is regularly observed together with several distinct bands with more advanced migration. The diffuse band pattern is reduced in Hiproly as compared with CI 4362 and Kristina. In the two latter varieties, the latest stage (10 % moisture stage) exhibits the strongest diffuse band state. The patterns from the salt-soluble extracts display essentially the same bands as those from water extract and could thus be regarded as a check on the efficiency of the extraction by water.

Different F_4 lines from crosses between Hiproly and normal barley varieties (Table 23) were analysed by polyacrylamide gel electrophoresis at the yellow-ripening stage. After sampling, seeds were immediately frozen until extraction. Some of the single endosperms investigated were likewise analysed with regard to amino acids in the different extracts and the remaining residues (Table 34). The most striking differences between the high-lysine and normal segregants were the approximate doubling of the water-protein fraction expressed as mol percentage of whole seed. This accords with the Kjeldahl analyses in Table 35 comparing Hiproly with its sister line. The salt-soluble fraction (Table 34) is not significantly changed because of the lys gene, whereas the remaining residual fraction, after extraction, is higher in the normal segregants. The lysine content (mol %) of the different fractions shows a slight tendency to be increased in the high-lysine segregants. More conclusive data are, however, obtained from the molar lysine to arginine ratio, the normal segregants displaying a significantly lower ratio, especially with regard to the water fraction. The molar aspartic acid to glutamic acid ratio is increased in the high-lysine lines owing to an increase in aspartic acid and a decrease in glutamic acid (Table 33). In the water-soluble and salt-soluble fractions (Table 34), however, the aspartic acid to glutamic acid ratio seems instead to be higher in the normal as

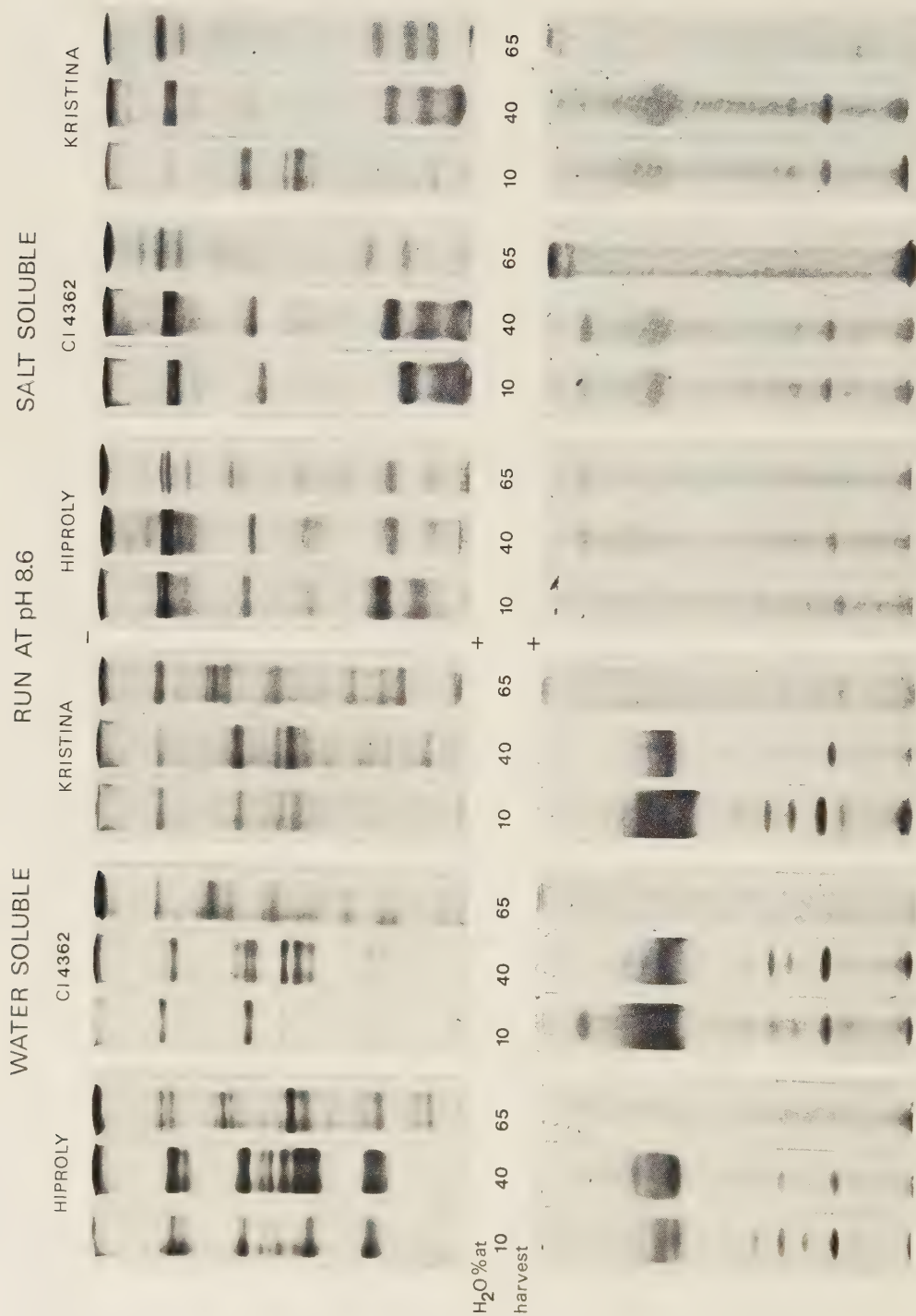


Fig. 32. Polyacrylamide electrophoresis towards anode (above) and cathode (below) with water and salt extracts from high-lysine (Hiproly) and normal lysine (CI 4362 and Kristina) barley endosperms. The endosperms display different developmental stages expressed as the approximate water contents at harvest for analysis.

Table 34. Amino-acid analyses of extracts from single endosperms segregating for the high-lysine gene

Fraction	n	Protein fraction %*	Lys mol %	Lys/Arg	Asp/Glu
<i>H₂O-soluble</i>					
Normal	5	6.6 ± 0.7	5.3 ± 0.3	1.07 ± 0.05	0.94 ± 0.10
High-lysine	4	13.1 ± 1.5	5.6 ± 0.3	1.33 ± 0.12	0.81 ± 0.01
<i>Salt-soluble</i>					
Normal	5	5.4 ± 1.0	3.7 ± 0.3	0.83 ± 0.20	0.55 ± 0.05
High-lysine	4	4.9 ± 0.9	4.2 ± 0.2	1.28 ± 0.27	0.49 ± 0.02
<i>Rest</i>					
Normal	5	88.0 ± 1.2	2.5 ± 0.2	0.77 ± 0.03	0.17 ± 0.02
High-lysine	4	82.4 ± 2.3	2.7 ± 0.1	0.84 ± 0.10	0.19 ± 0.01

* Based on Σ amino acids (mol %) of whole endosperm

compared with the high-lysine segregants, which is contrary to the previous statement. The residual fraction, however, which displays a very low aspartic acid to glutamic acid ratio, is quantitatively reduced in the high-lysine state, which apparently enables us to maintain the first conclusion with regard to the whole seed.

Two seeds with similar protein content, one high-lysine (Hily) and one normal, are compared in Fig. 33 (MUNCK 1972a) with regard to the electrophoretic patterns and the amino-acid composition of extracts. The increase with respect to the water-soluble fraction and the lysine to arginine ratio is confirmed further. The diffuse cathodic band pattern and water extract are reduced in the high-lysine (Hily) seed. The salt-soluble fractions display the remaining bands from the water extract that are especially marked in the case of Hily. In normal lines as regards the salt-soluble fraction, a pattern of three fast-moving bands are regularly seen as compared with a combination of four in the high-lysine segregants. The missing band in the normal line seems, however, to have been extracted already with the water extract. In general despite intensive screening, no new proteins have been ascertained to be formed owing to the presence of the high-lysine (lys) gene.

Changes in extraction properties, which are discussed in Fig. 32, might explain the qualitative differences obtained. To form a more definite conclusion, more penetrating basic research has to be carried out. The qualitative effects of the relative amounts of different protein fractions

are convincingly demonstrated in the amino-acid patterns and the amino-acid ratios. Thus the methionine to cysteine ratio of the unextracted residue (Fig. 33) is 3.24 for the high-lysine endosperm and 2.01 for the normal endosperm. Differences with regard to the formic acid-soluble fraction are small between the two samples, which accords with the results from several analyses (unpublished results).

The electrophoretic analyses of the water extract on a single endosperm basis was used for screening in the F_2 from a cross between Kristina and Hiproly (Fig. 34). Seventy-nine plants were studied and the lys lys lys phenotypes were selected. A ratio, not significantly different from 3:1, was obtained. These plants were checked with DBC:KP analyses displaying good agreement with the electrophoresis analysis. Separate endosperms of some of the plants that were heterozygotic were also studied employing electrophoresis (see Fig. 34). Three categories of plants were found in the F_2 from the Hiproly $\times$ Kristina cross. The high-lysine, normal homozygotic plants were slightly more variable than their parental counterparts, indicating the presence of possible modifier genes. Endosperms from heterozygotic plants displayed a wide range of patterns (Fig. 34), which could be due to the possible lys lys lys, Lys Lys Lys, Lys Lys lys and Lys lys lys gene combinations. It was not possible to distinguish between these tentative classes with electrophoresis performed on a single endosperm basis without considering the total protein content of individual endosperms. Because the

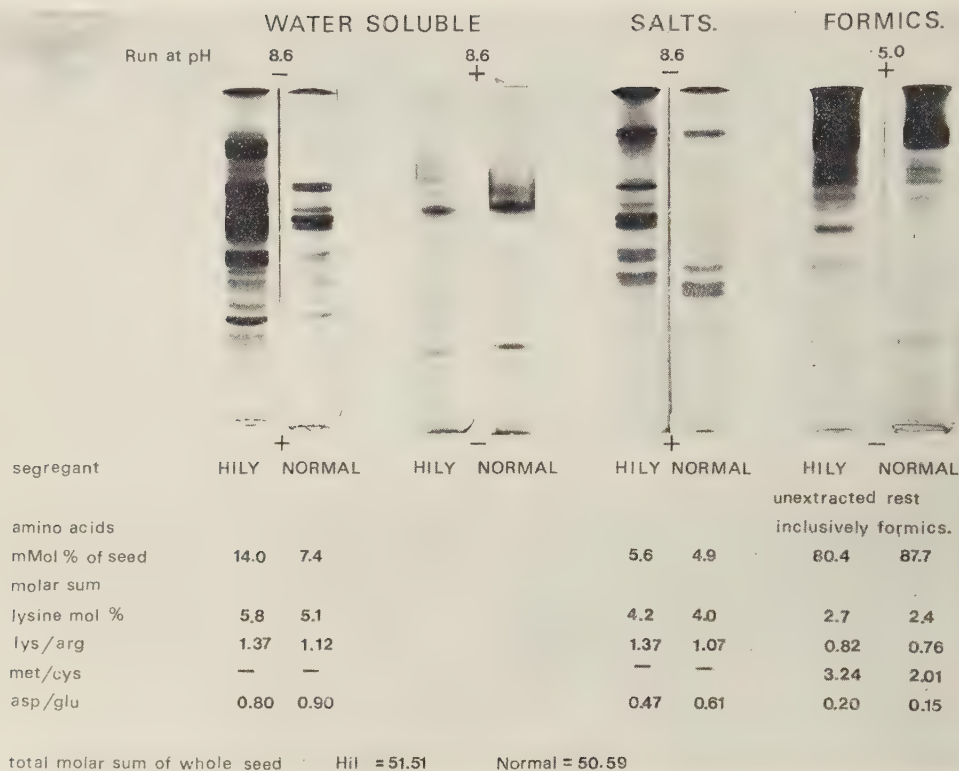


Fig. 33. A high-lysine (Hily) and a normal-lysine segregant for the lys gene analysed by polyacrylamide electrophoresis and amino-acid analyses of extracts from single endosperms. Yellow-ripening stage.

latter parameter varies by about 100 % when analysing endosperms from the same plant, the heterozygotic gene-dose response theoretically suggested will be overshadowed by environmental effects, which exhibits its influence also with regard to the water-soluble proteins, but at a much lower rate in comparison with the total protein content. However, single seed lysine analysis (Fig. 12) of the F_1 plant suggests complete dominance for the Lys "wildtype" allele.

Because the extraction of proteins might be affected by the drying of the seed endosperms from the yellow-ripening stage, Hipoly and CI 4362 harvested on August 4 were studied with and without drying to 10 % moisture content at room temperature (Table 35). Figures for the different fractions previously discussed are given as a percentage of total crude protein. It is seen that the difference in ethanol-precipitable water extract between the sister line and Hipoly decreases when passing the undried material to the

dried material. An appreciable amount of these proteins are denatured in Hipoly in contrast to CI 4362. The ethanol-soluble water extracts were decreased in both varieties, and the sodium chloride extracts increased by approximately the same amount. In CI 4362 less proteins were extracted with ethanol after drying, whereas the proteins in the residue were increased. In contrast to this, more proteins were extractable by ethanol in Hipoly after drying and no increase in the residual fraction occurred. Thus, the two lines behaved differently with regard to protein extraction before and after drying. It is evident that some of the proteins in the ethanol-precipitable water extract in Hipoly would fall in the ethanol fraction after drying.

To give more conclusive evidence of the extraction differences discussed, polyacrylamide electrophoresis was employed on freeze-dried extracts from the whole-water fraction, as well as from the water-ethanol-soluble and ethanol fractions.

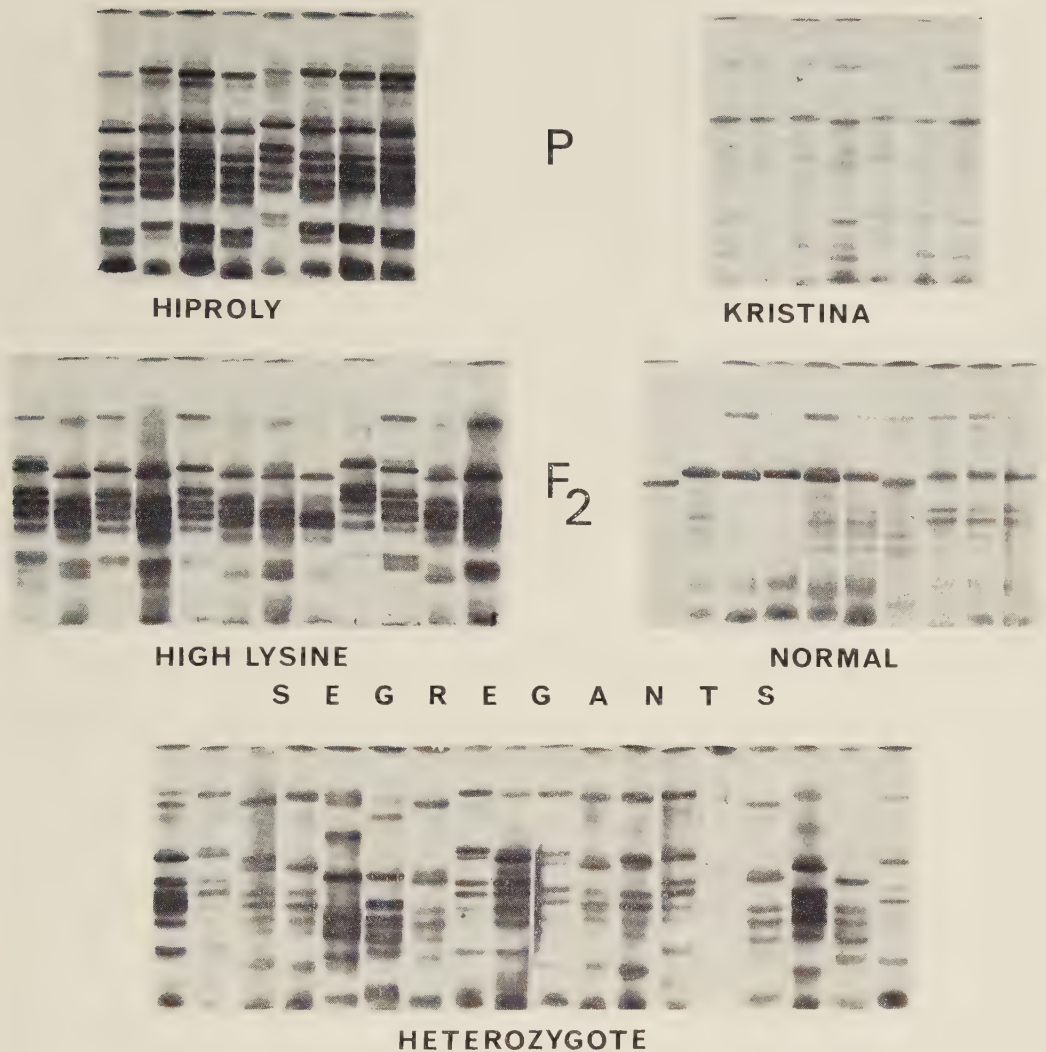


Fig. 34. Segregation for the lys gene. Polyacrylamide electrophoresis of water extracts from single endosperms in a high-lysine (Hiproly), and a normal-lysine barley plant (Kristina) is compared with a high-lysine, a normal-lysine and a heterozygous barley plant in the F_2 from the cross Hiproly ($\varnothing$) $\times$ Kristina (f). Yellow-ripening stage, anodic migration pH 8.6.

The freeze-dried extracts were redissolved in water and in 0.2 M formic acid (Fig. 35). It was found that freeze-drying already had a denaturing effect, and it was not possible to redissolve the proteins completely with water or with formic acid. In the whole-water extract more fast-moving proteins were extracted with formic acid both in Hiproly (H) and the sister line (S). There were no important varietal differ-

ences. In the water-ethanol-soluble and the ethanol freeze-dried fractions, there were no bands extracted with water (5; 6 and 9; 10). If these fractions are redissolved in formic acid, one strong and three faint bands were seen in Hiproly (7) in the ethanol-soluble water fraction; only traces were observed in the sister line. The same band combination, however, appeared in the sister line in the formic acid extract of the

Table 35. Protein fractions (% of total N $\times$ 6.25) of Hiproly and CI 4362 barley at yellow-ripening stage (4/8) with and without air drying

	Water extract		NaCl** (extract)	Ethanol* (extract)	Residue
	Ethanol* (precipitable)	Ethanol* (soluble)			
CI 4362	5.9	10.2	7.2	38.3	38.3
CI 4362 (dried)	6.1	9.5	8.0	36.6	39.8
Difference	+0.2	-0.7	+0.8	-1.7	+1.5
Hiproly	11.3	14.2	6.0	26.5	42.0
Hiproly (dried)	9.3	13.1	7.0	28.8	41.8
Difference	-2.0	-1.1	+1.0	+2.3	-0.2

* 70 % Ethanol

** 1.2 % NaCl

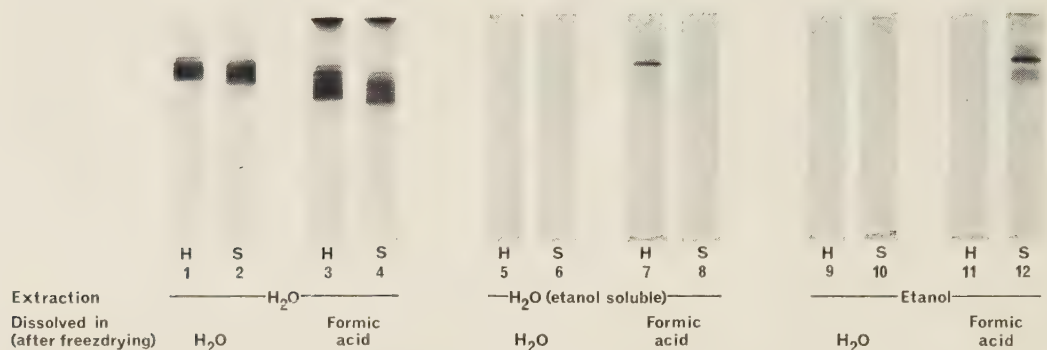


Fig. 35. Protein extraction differences between Hiproly (H) and its isogenic sister line (S, CI 4362) analysed by polyacrylamide electrophoresis of unripe seeds (Fig. 31; 19/7). Cathodic migration pH 5.0.

ethanol-soluble fraction (12), thus illustrating that similar proteins could be originally extracted by different solvents in the two lines, which confirms the varietal influences in extraction performance (Table 35).

3. Discussion

In Hiproly the protein bodies appear to be unchanged when compared with the sister line, i.e. as interpreted from investigations with the light and scanning electron microscopes (Fig. 25–29). This observation also accords with the fact that the ethanol-soluble protein fraction, which is comprised of the protein bodies, is

only slightly decreased in percentage of total protein in the Hiproly endosperm. In contrast to the conditions in barley, opaque-2 (o_2) and floury-2 (fl_2) maize present protein bodies that are greatly reduced in size. WOLF et al. (1967, 1969) found that protein bodies of normal maize, which were about 2 μ in diameter, could be observed in the light microscope, whereas in o_2 no such protein bodies could be discriminated. In the electron microscope, however, smaller bodies, with an average diameter of 0.1 μ , were observed in o_2 . The prolamine nature of the bodies was established by their solubility in ethanol. In fl_2 , WOLF et al. did not discover any protein bodies when employing conventional electron microscopy.

CHRISTIANSSON (1970), however, employing the

SEM technique with protein-body fractions obtained by ultracentrifugation, found highly variable protein bodies in fl_2 , ranging from 0.1 to 1.0 μ in diameter. The fl_2 protein bodies were irregular due to the layering of the protein in small thin sheets, which are also distinctly different from o_2 . The protein bodies were loosely organized into small clumps in the matrix-embedded condition. In the matrixed state both o_2 and fl_2 do not seem to be associated with as much matrixed glutelin as matrixed bodies in normal corn.

The electron micrographs obtained by CHRISTIANSSON in maize greatly resemble the previously discussed SEM studies in barley. Protein bodies in normal maize and barley endosperm seem to be of a similar size—about 2 μ . Those of barley are slightly irregular due to the attached matrix protein—possibly because they were not purified by ultracentrifugational separation. Thus, it is concluded that a reduction in the size of the protein bodies in o_2 and fl_2 is accompanied by effects in the matrixed proteins, which morphologically are especially evident in fl_2 . In the lys condition of barley, however, the size and shape of the protein bodies do not seem to be affected—the effect of the gene being mainly limited to the surrounding cytoplasm (matrix proteins). This effect is manifested as an altered solubility of the matrix proteins (Fig. 28, 29) and in an increased number of filamentous structures that are stainable with toluidine blue (Fig. 23A).

ORY and HENNINGSEN (1969) studied barley aleurone grains in the electron microscope. Protein bodies were isolated from a coarse-meal fraction (200 mesh), including the aleurone layer and other parts of the endosperm, and then separated by ultracentrifugation and studied in the electron microscope. Two types of protein bodies were observed with sizes varying from approximately 0.1 to 2 μ . In the protein-body preparation, the majority of the protein bodies stained uniformly with osmium and were contained within a single membrane with no other structural components. These protein bodies were similar to those of the central endosperm of maize that were reported by KHOO and WOLF (1970). The other type, mostly the larger particles, had a sub-structure of uniformly dark- and light-stained layers, which were attached to the protein bodies.

The protein-body preparation contained two acid hydrolases (ORY and HENNINGSEN 1969) and

beta-amylase (TRONIER and ORY 1970), as well as hordein based on results from an immunochemical method (TRONIER et al. 1971). Phytase activity in situ was found to be associated with aleurone grains, which were studied in sections from the aleurone layer. Definite conclusions from the cited experiments with regard to the localization of enzymatic activity are difficult to draw, except for phytase, because of the incomplete separation between the aleurone layer and the rest of the endosperm. It seems, however, likely that aleurone grains with different enzyme contents and functions should be expected to be found in the aleurone layer and in the central endosperm, respectively. Organelles containing important enzymes like phytase should thus be restricted to the aleurone layer, whereas the main function of the endosperm protein bodies should be to store proteins of prolamine nature.

From the extraction experiments with normal and high-lysine barleys, it is evident that the solubility of the different proteins with the Osborne technique is highly dependent on the ripeness of the seed and the lys character. When seeds are dried, some proteins are irreversibly denatured (Fig. 32, Table 35). Studies with the immature seed as regards protein composition as well as denaturation studies of individual proteins and protein mixtures are therefore essential to gain an understanding of the properties of the whole-dried, mature seeds.

The decreased amount of cysteine as a consequence of the presence of the lys gene is likely to imply a glutelin network that is less bridged with S—S bonds and thus changes the binding interaction of different proteins. Hence, it is likely that the lys gene changes the extractability of specific proteins per se compared with the same proteins in the normal state. Such a case is demonstrated in Fig. 35. Missing or extra bands in electrophoretic analyses of Osborne fractions should therefore be interpreted with caution and their counterparts should be searched for in other fractions.

JIMENEZ (1968) studied the Osborne fractions of o_2 , fl_2 and normal maize, as well as the double recessive genotype of o_2 and fl_2 . In o_2 the concentration of the albumin was doubled and the non-protein nitrogen tripled in comparison to normal maize. In fl_2 and the double mutant, only the non-protein nitrogen fraction increased. The

increase of the albumin fraction of o_2 was mainly due to a characteristic change in the amount of a specific band in starch-gel electrophoresis. In Hiproly a similar, characteristic increase of the albumin fraction was obtained with regard to five anodic bands — all with a medium migration velocity in polyacrylamide electrophoresis (Fig. 33). No major effect was observed in Hiproly on the low-molecular nitrogen fraction in contrast to o_2 and fl_2 .

JIMENEZ's (1968) studies indicated that o_2 differed only quantitatively from normal maize in the albumin, globulin, and glutelin fractions. In the zein fraction, o_2 and the double mutant lacked three bands that were present in normal maize. The fl_2 mutant differed both quantitatively and qualitatively from o_2 and normal maize in all four fractions. With regard to the lys gene in barley, no safe conclusions with respect to qualitative differences in the electrophoretic fractions could be drawn from the material previously presented, although apparent qualitative differences, compared with normal barley, exist in the water- and salt-soluble fractions.

The main factor responsible for the high-lysine content in o_2 (JIMENEZ 1968) was the shift of the zein to glutelin ratio from 55:30 in normal maize to 25:50 in o_2 because of a decrease in zein. In fl_2 a similar shift of this ratio was not as evident as in o_2 because both the zein and glutelin fractions, on an endosperm basis, decreased instead of only the zein fraction.

Recent studies in maize (MISRA et al. 1972) have revealed six new, high-lysine mutants all displaying changed starch characters. These are sugary-1 (su_1), shrunken-1 (sh_1), shrunken-2 (sh_2), shrunken-4 (sh_4), brittle-1 (bt_1), and brittle-2 (bt_2). In addition, a new, high-lysine opaque mutant has been found (McWHIRTER 1971) — opaque-7 (o_7). Although the mutant genes are located at least on three different chromosomes, they exert a similar effect on endosperm composition comparable to o_2 and fl_2 . Albumins, globulins, and a glutelin fraction were all more or less increased, whereas zein was reduced. When o_2 and bt_2 were combined, lysine was enhanced, differing from the double mutant o_2fl_2 , where no synergistic effect was observed. MISRA et al. (1972) concluded that different pathways leading to reduced zein synthesis may exist in the floury and starch-modifying mutants with high-lysine concentrations.

INGVERSEN and KØJE (1971) studied dried seeds from four high-lysine barleys including Hiproly, KVL system 468 (TOFT-VIUF 1972) and two mutants, 29 and 86 (DOLL 1971). The results from polyacrylamide electrophoresis revealed both quantitative and qualitative differences in the albumin+globulin pattern between the high-lysine lines and a normal variety, Carlsberg II. Some of these bands stained heavily with Acilane Orange G, indicating that they might have a high content of basic amino acids (lysine). Further separations with Sephadex and polyacrylamide electrophoresis revealed very high-lysine contents in the specific albumins, which were increased in Hiproly (INGVERSEN and KØJE 1972, pers. comm.). In none of the lines examined by INGVERSEN and KØJE (1971) were differences found in the hordein fraction. With regard to the qualitative changes that have tentatively been demonstrated, this has to be verified with more appropriate separation techniques of non-dried endosperms.

Only a few studies of the protein synthesis in o_2 maize have been made. MURPHY and DALBY (1971) made comparative studies of the Osborne fractions in developing o_2 endosperm and normal maize. The total saline-soluble nitrogen per endosperm of o_2 increased continuously during the period studied, which was in sharp contrast to the normal endosperms, which were much more constant. In the studies with Hiproly and the sister line (Fig. 31), it is evident that the water-soluble proteins also increase especially in Hiproly compared with the normal barley material. From the electrophoretic pattern, it is seen that a series of bands which appear early vanish in later stages, whereas several bands that appear late in the developmental period are newly synthesized. The latter proteins are markedly increased in Hiproly and in segregants homozygous for the lys gene. The increase of these water-salt-soluble proteins is paralleled with a linear increase of prolamines of barley, which possibly signifies that these two protein groups are functionally dependent on each other during synthesis.

MURPHY and DALBY (1971) confirmed that the zein synthesis of o_2 is drastically reduced compared with normal maize. The difference in the synthesis of the alcohol-soluble proteins between Hiproly and the sister line is much less, which points out major differences between the o_2 and lys genes.

According to DALBY et al. (1972), the o_2 gene in maize must be considered to be a regulatory gene and not a structural one. DALBY and DAVIES (1967) and WILSON and ALEXANDER (1967) found highly increased ribonuclease activity in o_2 when compared with normal maize. The maize endosperm ribonuclease has a base specificity for purines exceeding that for pyrimidines. DALBY et al. (1972) discuss the possibility of a preferential destruction of purines with anticodon sites in t-RNA over the corresponding pyrimidine-rich sites, which might affect the relative ratios of the different proteins. This possibility was examined by measuring the amino-acid acceptor activity of t-RNA fractions isolated from 22-day normal and o_2 endosperms. There appears, according to Dalby et al., to be more inactive RNA in the o_2 t-RNA preparation; but the preferential destruction hypothesis was not verified through the amino acid t-RNA concentrations obtained. It could, however, not be dismissed that some t-RNA species, when split at the anticodon site, are still capable of accepting amino acids but not transferring them at the ribosomal level. In addition, DENTČ (1969) pointed out differences with regard to the activity of synthetase preparations between normal and o_2 towards a particular endosperm t-RNA preparation. DENTČ (1970) finally found that there was a greater incorporation of lysine in the presence of ribosomes from the o_2 genotype than with the system with ribosomes from the normal maize type. Incorporation was independent of the origin of the t-RNA used.

We can thus tentatively conclude that the presence of different ratios of messenger RNA's seems to be directly involved in the o_2 to normal difference in maize. DALBY and CAGAMPANG (1970) suggested, on the basis of ribonuclease data, that the o_2 gene in maize was active only during the first 16 days after pollination as far as ribonuclease is concerned. DALBY et al. (1972), however, found a deviating pattern for two o_2 inbred lines out of nine studied. Here, differences in ribonuclease activity between the normal and mutant lines occur later in development; but in contrast to the other seven lines, the activity persists to maturity. The two deviating o_2 lines were not completely floury, but contained horny regions in the endosperm. The precise role of ribonuclease in the o_2 gene complex still remains to be defined.

In the present investigation the approach has been to trace the effects of the lys gene of barley from the phenotypical level and to compare it in connection with the maize protein mutants. Although "the grain proteins ... are much closer to the gene than most characters for which the plant breeder is accustomed to selecting" (NELSON 1970), the high-lysine characters might constitute the phenotypical expression of genes which are not necessarily directly involved in the synthesis of the storage proteins. The fundamental effects of these genes are still not revealed, but, on the other hand, we are striving with a lot of defined apparent effects, that we do not know whether they are primary or secondary. Although it is not necessary to know all the factors behind these specific genes to obtain a breakthrough in this branch of plant breeding, it should be of fundamental interest, at least for plant-breeding forecasts to reach a general insight into the regulatory mechanism of the protein synthesis in cereal endosperm. One of these fields for future development is the relationship between the biochemical composition and the ultrastructure of the endosperm cell. A suitable model already exists in the studies by VON WETTSTEIN and ERIKSSON (1963) as regards chloroplast development in normal and mutant barleys. Changes in chloroplast structure could be evoked not only by mutations changing the synthesis of chlorophyll and the accessory pigments, as carotenoids, but also through a loss in the capacity to produce an essential metabolite, like aspartic acid, or through mutations affecting electron transport. There are also genes which directly affect the structural development of the plastids either through nuclear genes or through DNA-specific genes for the chloroplast (see review by NELSON 1967).

The effects of the opaque-2 and floury-2 genes on the protein bodies seem to be more primary than the soft character (floury) of their endosperms. The latter characters could be completely modified in o_2 . The fact that new o_2 mutants could be induced that display a high-lysine content favours the hypothesis that the mutational event affects both a "soft endosperm gene" and a "protein-regulating gene" that are in close proximity to each other. The fact that, non-opaque high-lysine o_2 's without altered amino-acid composition could be frequently obtained by crossing indicates that these effects may be due to modi-

fier genes and not to crossing-over between a "soft endosperm gene" and a "protein-regulating gene". It is interesting to note that an analogous condition is tentatively prevalent in *lys* barley displaying a hard endosperm compared with normal barley lines, which also seem to be modified without directly affecting the amino-acid composition.

Another interesting problem that should be solved is to gain an appreciation how very hydrophobic proteins, such as the prolamines, can, at all, be synthesized and transported in the endosperm cell. From ultrastructural studies previously discussed, it seems as if the endoplasmic reticulum and the Golgi apparatus function as a synthesis and transport mechanism for the prolamines until they are stored in the protein bodies. Prolamines, however, also leach out into the matrix, where the rather specialized glutelins are also synthesized. Experimental data from this investigation tentatively indicate a major shift in the metabolism of the endosperm at about 60 % water content from which stage the prolamines are particularly intensively synthesized paralleling a termination of the starch synthesis in the amyloplasts. The role of the water-soluble proteins, which especially increase during this period in *lys* barley and in o_2 maize, has yet to be defined, but it is likely that they are involved in the synthesis of the prolamines either as structural elements close to the membranes or in a tentatively "detergent" action to maintain the prolamines soluble during transport. If this were so, a change of the composition and amounts of the albumin fraction could impair prolamine transport and synthesis.

Still another field of research is the study of the intermediate amino-acid and carbohydrate metabolism in the high-lysine mutants. JOLIVET et al. (1970) found in a radioactive labelling experiment with young leaves of o_2 and normal isogenic maize that there were fewer carboxylic acids as well as amino acids related to the tricarboxylic acid cycle (malate, citrate, aconitate, succinate, aspartate, threonine, alanine, and glutamate) in the case of the o_2 mutant, whereas the opposite was observed in the case of glycerate, serine, and glycine. These differences suggest that o_2 is also active in leaf tissue. SODEK and WILSON (1970) found that normal maize endosperm is able to convert lysine into glutamic acid and proline, whereas the endosperm of o_2

is unable to do this until at later developmental stages.

It may be alternatively hypothesized that genes conditioning the high-lysine endosperm in cereals could primarily affect the rate of the basic intermediate metabolism. Such a change may more easily be expressed in the endosperm than in the leaf or the embryo because the former is a more specialized tissue which is tentatively more sensitive to a radical rearrangement in amino-acid and protein metabolism.

V. Relations between nutritional quality, morphology, and chemical composition of the seed

The cereal seed is obviously morphologically differentiated into compartments that also display great chemical differences. It is therefore important to understand the nutritional consequences of this differentiation in improving cereals. The wheat dry-milling process enables a suitable fractionation, which was already utilized by OSBORNE (1924) in rat growth studies.

In Table 36, the chemical composition of seven milling fractions is shown as well as that of whole-wheat meal. In all, 42 fractions were obtained from a modern grain mill (Pååls Bröd, Gothenburg). The crude-protein content is highest in the embryo (P42, 30.1 %) compared with 10.0 % in the whole meal and with 12.8 to 19.3 % in the aleurone region (P37–P41). In the endosperm centre (P10), protein content (10.0 %) is equal or less than in the whole meal, whereas intermediate fractions (P12) contain as much as 14.2 % protein. It is also seen from Table 36 that lysine g/16 g N, crude fibre %, ash %, Ca %, K %, Fe mg/100 g N, P % and resorcinols (relative units) are greatly increased in the outer part of the endosperm (P37–P41) as compared with the inner fractions (P10–P12). The embryo contains more lysine and much less fibre, Ca, Fe, and alkylresorcinols than the aleurone fractions (P37–P41). Lysine g/16 g N is highest in the endosperm in the P37 fraction (6.01 %) and gradually decreases to 4.86 % in the outermost parts of the seed (P41). P37 is also comparatively low in fibre and resorcinols, which in combination with the high-lysine content might be advanta-

Table 36. Chemical composition of wheat milling fractions

Milling fraction	Crude protein (%)	Lysine (g/16g N)	Fiber (%)	Ash (%)	Ca (%)	K (%)	Fe (mg/100g)	P (%)	Resorcinols (rel. units)
P10	10.0	2.31	0.10	0.24	0.016	0.12	1.67	0.05	14
P12	14.2	3.11	0.90	1.80	0.012	0.36	5.48	0.33	39
P37	19.3	6.01	4.70	3.52	0.057	0.75	8.39	0.68	124
P38	14.8	5.88	9.30	3.93	0.075	0.87	12.46	0.66	382
P39	14.0	5.66	11.60	5.10	0.084	1.19	18.43	0.86	516
P41	12.8	4.86	12.75	6.44	0.073	1.45	15.18	1.23	538
P42 (embryo)	30.1	7.70	2.40	4.55	0.034	0.94	8.41	0.95	40
Whole meal	10.7	3.34	1.95	1.28	0.032	0.32	3.35	0.27	90

geous with respect to nutritional quality. The inner fraction (P10) is much lower in lysine (2.31 %) than the whole-wheat meal (3.34 %).

The fractions discussed in Table 36 were fed to rats and mice, which results are presented in Fig. 36 and 37. The nitrogen retention in mice (NR) and the net protein utilization in rats (NPU), which animals were fed these diets at a 9.4 % crude-protein level, are comparatively well related (Fig. 36). A methionine-supplemented casein diet (C) as a control was also included. There is a marked negative relationship between nitrogen true digestibility for rats and the crude-fibre content. The rat digestibility values were used as an estimate to calculate the consumed digestible lysine in the mouse trial (Table 37), assuming true digestibility coefficients proportional to that of nitrogen as comparable in the two kinds of animals. The high lysine P37 fractions, low in resorcinols, display a high nitrogen utilization in rats equal to the embryo (E) diet; in mice, P37 exceeds the latter diet. If the consumed digestible lysine is considered in relation to gain in protein in the mouse trial, the "aleurone" diets (P37–P41) show a continuously decreasing gain in protein with the decreasing consumption of lysine. The other diets consist of the high-lysine embryo (E) on the upper side of the P37–P41 regression and of the low-fibre, low-lysine diets P10, P12 and whole wheat (W) on the lower side. The digestible lysine in the embryo is thus utilized less effectively and P10, P12 and W more effectively than expected compared with the P37–P41 relationship. It is also seen in Fig. 37 that the consumption of alkylresorcinols is greatly increased, especially in P38–P41. At the same time, the gain in protein is

reduced. Because of very strong associations ($r=0.98-0.99$) between crude fibre and digestibility on the one hand and alkylresorcinols on the other, it is not possible in this experiment to statistically differentiate these effects from each other.

Gain in protein is well correlated with gain in fat for mice. It is interesting to note that P41, which has a slightly higher intake of digestible lysine than P10 and displays a similar gain in protein, exhibits a strongly depressed gain in fat when compared with P10. This could be due to a depression in available carbohydrates correlated with the crude-fibre content. Another explanation might be founded on the great differences in alkylresorcinols between P41 and P10 (Fig. 37). The high content of alkylresorcinols in P41 could, according to this hypothesis, function as a fat-absorption inhibitor. The effects previously studied in mouse trials (Tables 8 and 9) feeding rye, and resulting in a low gain in fat as compared with wheat, are in agreement with such a possibility. Differences in carbohydrate and protein availability could, however, also explain these differences between the two cereals. The studies by WIERINGA (1967), in which rats and swine were fed purified alkylresorcinols, indicate that specific alkylresorcinol effects may be common also in this experiment, although they could not be differentiated from the crude-fibre digestibility complex.

From the experiment discussed, we can conclude that the amino-acid balance of the cereal seed, from the nutritional point of view, is likely to be improved by an extension of the aleurone region, as discussed in Chapter III. Due consideration has, however, to be taken to the decrease

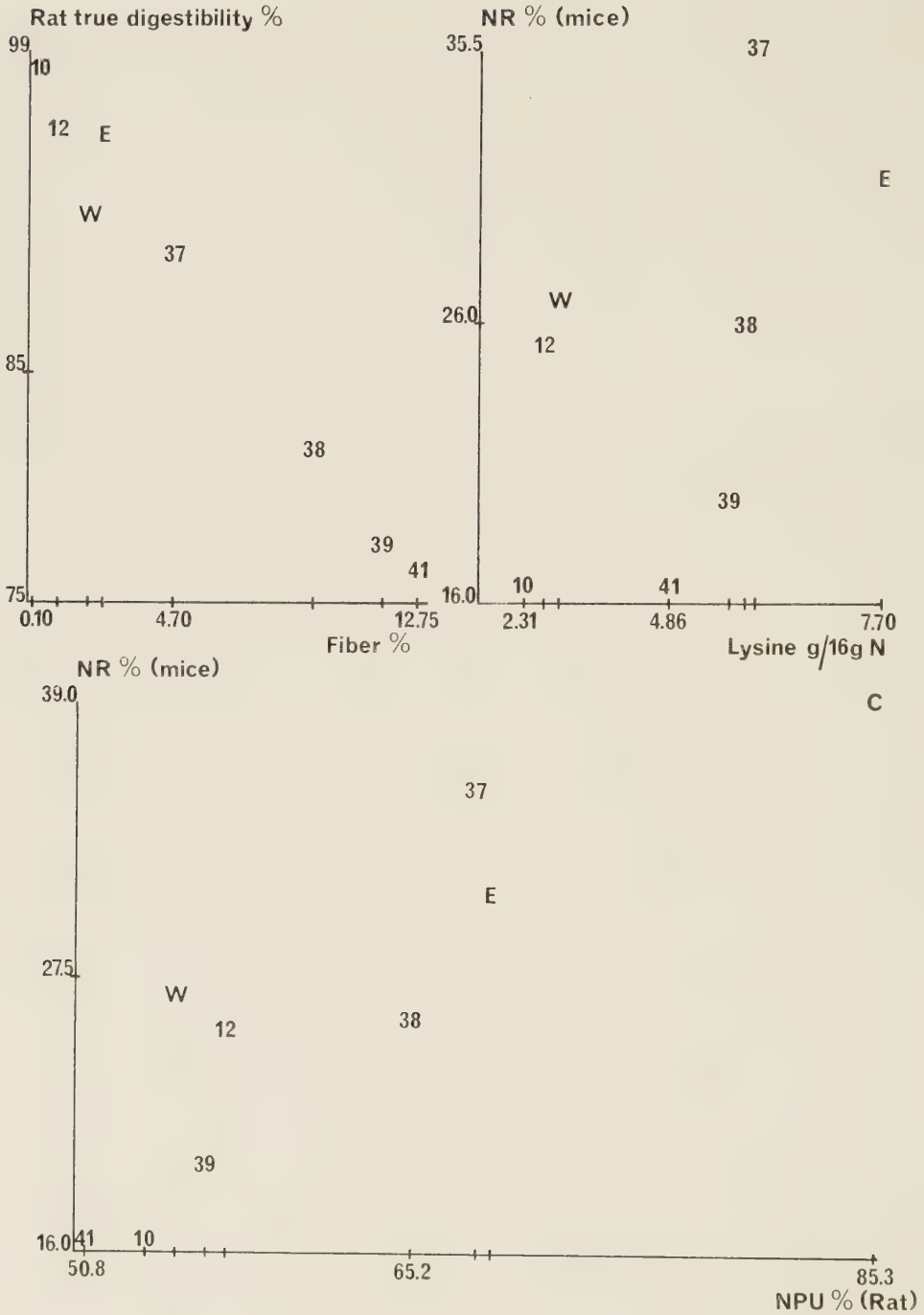


Fig. 36. Commercial wheat mill fractions fed to mice and rats at 9.3 % crude protein in the diet. Rat true digestibility (%) is related to crude fibre (%) and mouse nitrogen retention (NR, %) to lysine (g/16 g N). Rat net protein utilization (NPU, %) to lysine (g/16 g N). Rat net protein utilization (NPU, %) is compared with mouse nitrogen retention (NR, %). Fraction numbers 10–41, E (embryo), W (whole-wheat meal) and C (casein) were fed.

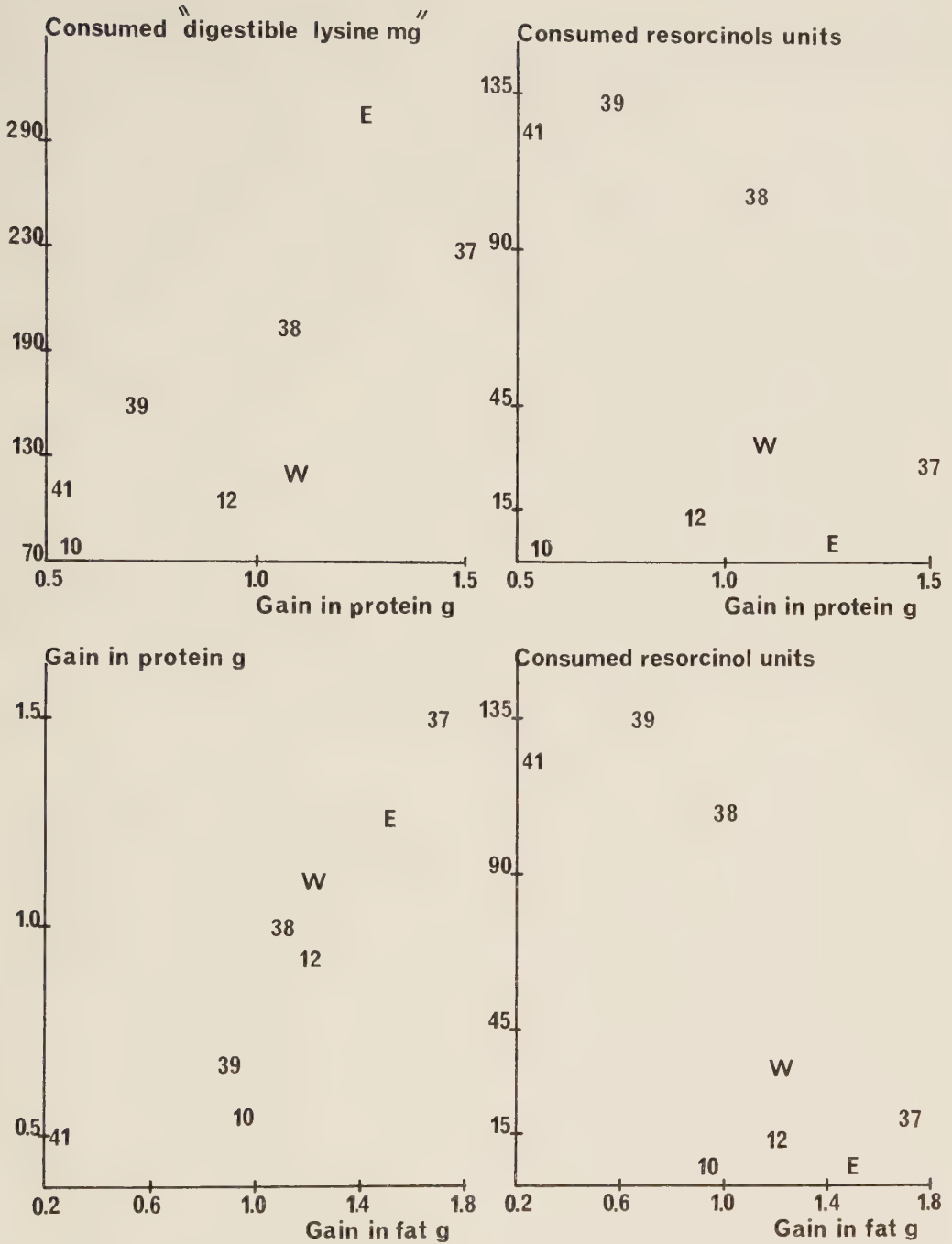


Fig. 37. Wheat mill fractions fed to mice. Diets as in Fig. 36. Relations between carcass parameters as gain (g) in fat and protein, consumed resorcinols (relative units) and estimated consumed digestible lysine (mg).

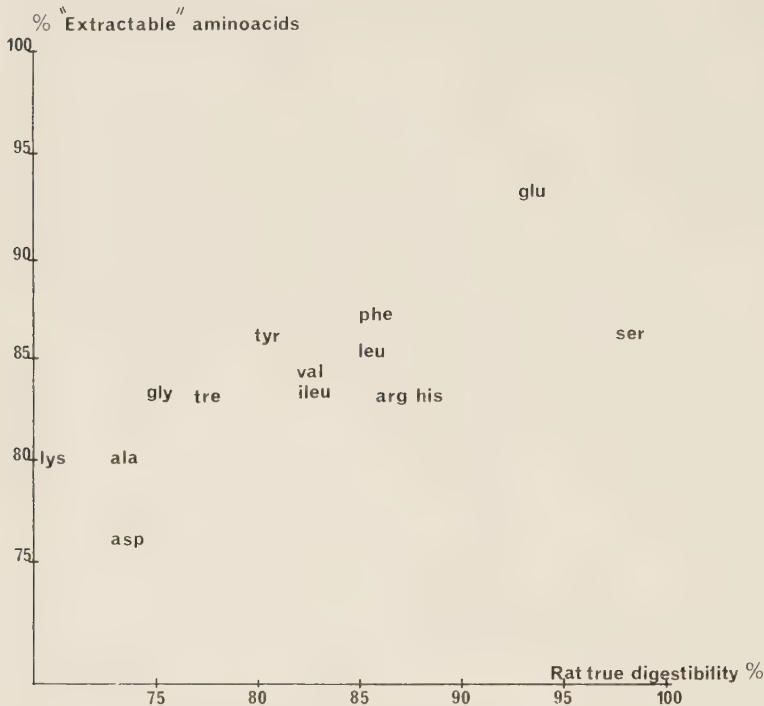


Fig. 38. Rat true digestibility (%) of individual amino-acids related to "extractability" (%) in free or protein bound form (according to the Osborne extraction procedure in Fig. 30) for the respective aminoacids. Normal lysine barley sample.

in digestibility, especially considering lysine which is prevalent in the aleurone region, as well as to the presence of deleterious factors such as alkylresorcinols and phytic acids. Tannin associated with the seed coat also impairs protein and energy digestibility, which is especially accentuated in sorghum.

The true digestibility of the individual essential amino acids is a more important factor in barley, which has a multicellular aleurone layer, than in, e.g., maize and wheat, which display a unicellular aleurone layer. It could be concluded from the studies with wheat-meal fractions that the nutritious proteins in and around the aleurone layer are partly trapped in morphological structures that are difficult for the animal to digest. These proteins also constitute great problems for the chemist as regards extraction (MUNCK et al. 1971; MUNCK 1972a), which is indicated by the fact that after an Osborne extraction series, the amino-acid patterns of the residual proteins con-

tain large amounts of essential amino-acids, among them lysine (Fig. 30). In a normal reference barley diet (Fig. 38), the relationship between rat true digestibility percentage for individual amino acids and percentage extractable amino acids with the Osborne procedure followed by hydrolysis is demonstrated (MUNCK 1972a). It is evident that those amino-acids that are easily extracted (mostly in protein-bound form) with the modified Osborne extraction series, such as glutamic acid and serine, are also more easily digested by the rat, whereas lysine, alanine, and aspartic acid, which are poorly digested, are also difficult to extract. It is thus reasonable to conclude that relationships between seed morphology, protein extraction performance, and amino-acid availability, are important for the proper nutritional utilization of the cereal seeds. These relationships obviously open up new possibilities for chemical screening methods for estimating amino-acid availability.

Table 37. Feeding trials with mice and rats comparing Hiproly and a reference (mixture of four naked, high-protein normal barley lines)

	Protein (%)		Lysine (g/16 g N)	Mouse* growth test				Rat nitrogen balance test (EGGUM) (n = 5)				
	Diet	Sample		Gain per animal		Crude protein (g)	Fat (g)	Protein consumed (g)	NR (%)	TD (%)	BV (%)	NPU (%)
				Total weight (g)	Dry matter (g)							
Hiproly reference	9.40	16.81	8.9 ± 0.7	2.84 ± 0.50	1.70 ± 0.14	0.63 ± 0.30	5.2 ± 0.4	32.7 ± 1.3	85.2	76.0	64.8	
	9.33	17.25	7.8 ± 0.5	2.53 ± 0.30	1.43 ± 0.11	0.55 ± 0.18	5.0 ± 0.2	28.4 ± 1.7	83.2	71.1	59.2	

* Individually fed, male Swiss × CBA, 14-day period, 7–8 animals per diet

Low-lysine prolamines building up the protein bodies in cereals seem to be readily available as indicated by studies with barley in vitro (MUNCK 1964a, d) and in vivo (EGGUM 1970a); SCHILLER and OSLAGE 1970). The true digestibility of nitrogen increases with increasing crude-protein level in the seed: lysine in percentage of protein, as well as the biological value, simultaneously decrease because of an increased contribution by the prolamines to the total crude protein of the seed. In swine experiments, THOMKE and FRÖLICH (1968), THOMKE (1972d) obtained positive effects of increases in barley protein owing to variety or cultivation conditions, provided the protein supplementation through soy was kept marginal. The productive value of the increased amount of barley protein seemed to be less than half of that of soy protein.

In the investigations of the high-lysine barley, the first experiments on mice and rats (MUNCK et al. 1970) were made comparing Hiproly with a reference mixture of four naked, high-protein normal lines (Table 37). Lysine g/16 g N was 4.08 for Hiproly and 3.13 for the reference diet mixture. At similar protein intakes in a mouse growth test, nitrogen retention and crude protein gain were increased in Hiproly barley. In rat trials, true digestibility was slightly increased in Hiproly, whereas the increase in the biological value was more pronounced when compared with the reference diet. The true digestibility of lysine was increased in Hiproly as compared with the reference diet. In Fig. 39 these diets are compared with respect to the true digestibility of 17 amino acids. The availability of lysine and methionine is slightly more than 75 % compared with 90–95 % for glutamic acid and serine. The least available amino acids – such as lysine, methionine, alanine, aspartic acid, glycine, treonine, and tyrosine – display a greater increase in availability in Hiproly than the best available amino acids – histidine, glutamic acid, and serine – when compared with the reference diet. The increased availability pattern thus reflects the enhancement of the water-soluble, lysine-rich proteins (Chapter IV), which should be easily digestible as regards Hiproly. These results on rat digestibility should be relevant even for swine, judged from further investigations by EGGUM (1970b). The enhancement of NPU in rats is comparable to that of the nitrogen retention in mice (Table 37). These differences in nutritional performance in

FIGURE

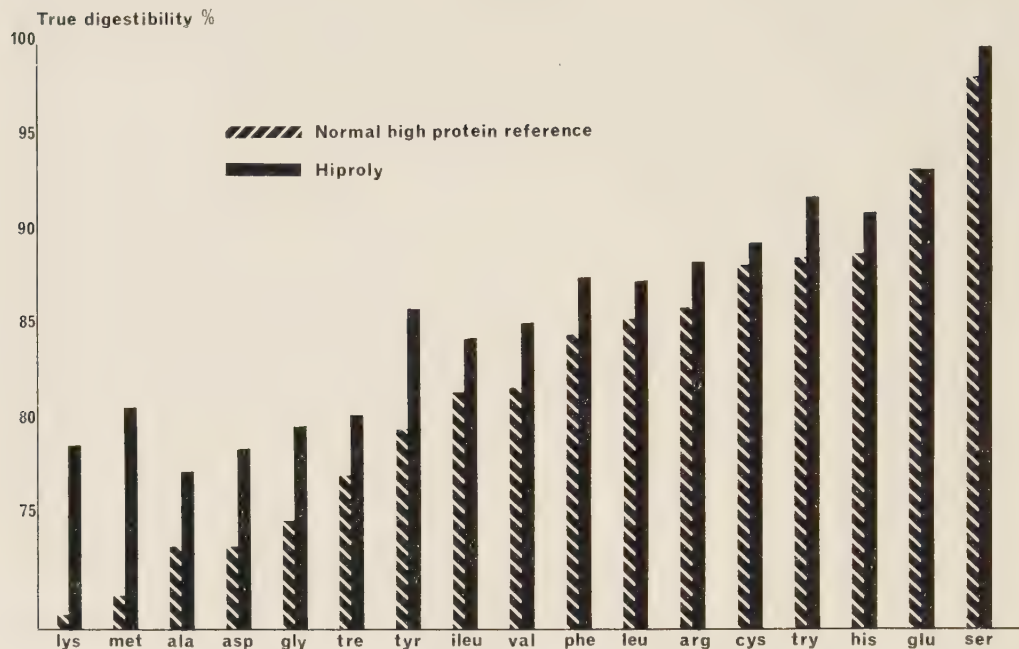


Fig. 39. A comparison of a high-lysine, high-protein barley (Hiproly) with a normal-lysine, high-protein reference with regard to rat true digestibility (%) of individual aminoacids.

mice and rats are small, although there was a considerable increase in lysine.

From the preceding discussion in Chapter I, it is, however, evident that young mice and rats immediately after weaning are much less sensitive to dietary depressions of lysine compared with piglets at a similar developmental stage. From the cited experiments and from Table 7, it could be concluded that lysine in diets of about 4 g/16 g N is hardly the limiting amino acid in mouse and rat trials with 9.4 % crude barley protein in the diet. Since the increase of lysine with the lys gene is combined with an increase in other important amino acids, such as threonine, methionine and valine, which could be limiting in the high-lysine barley diets, the biological value and the nitrogen retention may still be indirectly correlated with the increase in lysine in mouse and rat trials. The same conclusion is valid for the wheat-milling fraction study previously presented.

In order to test the nutritional quality of the

high-lysine morphological deviates showing no starch-protein adherence (Table 23) in contrast to high-lysine controls, which displayed a strong aggregation between starch grains and protein, rat studies were performed (Table 38). High-lysine lines deviating with regard to DBC (low value) were also tested. All of these lines were derived from the same F_2 plant, homozygous for the lys gene. A related normal lysine control was included. In Table 38, it is seen that NPU is considerably increased up to 10 % in the high-lysine lines compared with the normal control line. True digestibility is slightly increased in all high-lysine lines compared with the normal control. The starch-protein adherence trait of the high-lysine controls does not affect true digestibility, biological value and net protein utilization as compared with the high-lysine lines with normal morphology. The low DBC deviates display, however, a low biological value, which probably reflects a lower availability of, e.g., lysine in these samples. The true digestibility of nitrogen

Table 38. Nutritional value of different F₄ selections for rat

	Protein (%)	Lysine (g/16 g N)	SF (%)	BV (%)	NPU (%)
Normal control	12.6	3.25	84.0	71.2	59.8
High-lysine control	13.4	3.97	86.7	79.7	69.2
	12.4	4.13	85.1	80.6	68.7
High-lysine, low DBC	13.1	3.93	86.7	76.1	66.0
	13.0	3.88	86.9	73.5	63.9
High-lysine, normal morphology	12.8	4.10	86.0	79.6	68.4
	12.2	4.09	86.0	78.6	67.7
	12.5	4.02	85.4	78.6	67.1
Normal control	14.6	3.39	88.5	68.1	60.2
High-content coarse meal	13.8	4.08	87.9	76.0	66.8
Low-content coarse meal	14.8	3.93	86.7	75.6	65.7

is, however, not hampered because of the low DBC. It was concluded from the previously cited results that these meals were especially coarse after milling. To confirm the effect of milling performance in rat trials, a family with 60–65 % differences in coarse meal (above 120 mesh) between the high-lysine lines was compared with a normal lysine control. No differences with respect to TD, BV, and NPU were recorded between the two high-lysine lines with different milling performances. It is thus not likely that the milling differences *per se* are responsible for the low BV's and NPU's in the low DBC deviates that are high in lysine (Table 38).

Further scrutiny in the study of other probable limiting amino acids in high-lysine barley diets for rats and mice, as well as confirmations from feeding experiments with swine and chickens, are needed. Human investigations should also be performed, for barley is used as food by about 200 million people in arid countries in the Middle East and South America. It is reasonable to conclude, however, that lysine should still be the major selection goal: first, because it is needed by especially young farm animals and human beings; secondly, because its increase is positively correlated to the other important essential amino acids.

The best high-lysine barley lines display a NPU

in rat tests that is equal to opaque-2 and superior to floury-2 (EGGUM pers. comm.). VERON (1968) showed that rat growth is increased 3.6 times on an opaque-2 diet compared with an isonitrous normal maize diet. Digestibility was not changed. Addition of lysine and tryptophane to an opaque-2 diet only slightly increased weight gains. Floury-2 was inferior to opaque-2 in supporting rat growth (VERON 1968; NELSON and MERTZ 1972).

With regard to opaque-2 these results were verified in swine feeding tests (CROMWELL et al. 1967a, 1969). In chicken tests, the favourable effects of the opaque-2 gene was evident only after a methionine supplementation, as compared with normal maize (CROMWELL et al. 1967b). Poultry has a strong requirement for sulphur amino acids. In studies involving the content of metabolizable energy for chickens (GIPP et al. 1968), floury-2 was markedly inferior to opaque-2. It was not established whether this effect was associated with the floury-2 gene *per se* or if it was due to differences in the genetic background. The increase in methionine in floury-2 compared with normal maize and opaque-2 should, however, be especially advantageous for the nutritive value of the protein to poultry.

BRESSANI et al. (1970), among others, convincingly demonstrated the superiority of opaque-2 maize over normal maize in studies with young

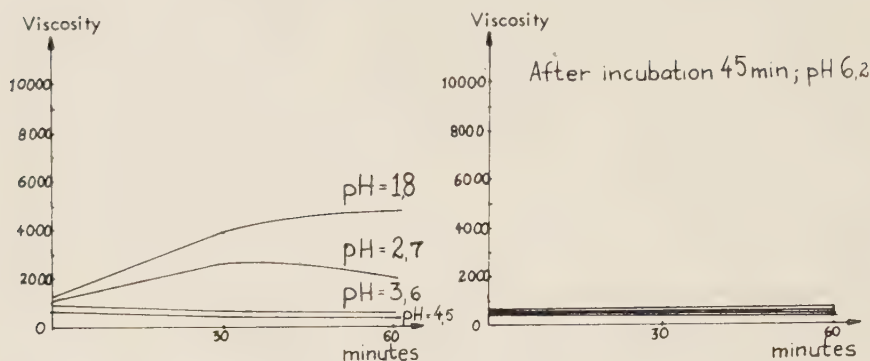
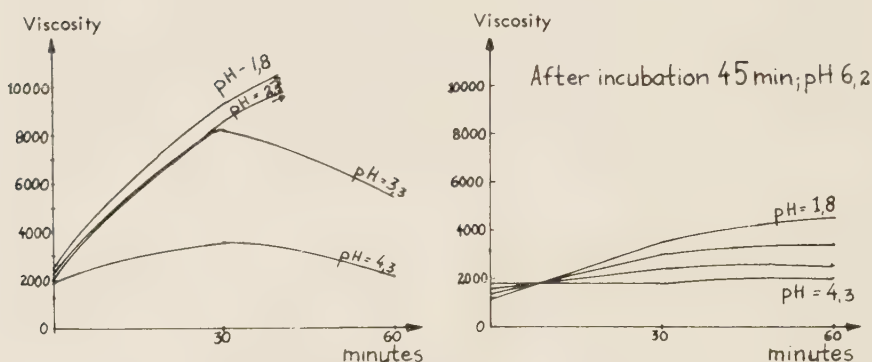
Barley variety Kristina.Barley variety Birgitta.

Fig. 40. Differences in pH-dependent viscosity (cps) of meal suspensions between barley varieties Birgitta and Kristina grown in Sweden. In two experiments, 92 g of meal was mixed with 200 ml diluted HCl. In the other two experiments, 45 minutes pre-incubation at pH 6.2 was carried out before adding HCl. Total fluid volume was 200 ml. Viscosity was measured at different time intervals in a Brookfield synco-electric viscometer at 25°C, after shaking for 1 minute.

children. Opaque-2 maize gave nitrogen retention values reaching 90 % of that of milk. The new maize mutants thus have a great potentiality in combating diseases due to protein deficiency in man.

Various less well-defined dietary factors in cereals, other than those usually dealt with in nutrition, interact with the digestive system and the microbiological flora. These factors, of great importance for health of the human being and economy in rearing farm animals, are most

drastically exemplified by the syndrome idiopathic steatorrhoea (coeliac disease) in man, resulting from malabsorption of particularly fats and minerals as a result of sensitization of the intestinal mucosa to gluten (BRONSTEIN et al. 1966). This severe disease may also be associated with skin lesions in the form of dermatitis herpetiformis (FRY et al. 1969). These patients are treated with gluten-free diets.

In the cereal inventory (Chapter II) it was found that the enzyme content, expressed as

alpha amylase, in barley exerts a positive effect on mouse growth. The slight changes in the gross chemical composition in barley owing to high enzyme content could not explain the response in nutritional performance. Nutritive quality of oats was not positively affected by a high content of alpha amylase. THOMKE (1972c) found a positive effect of late harvest in Sweden in feeding trials with chickens.

As was previously mentioned, viscosity is especially important for birds, which have a low water content and which lack carbohydrases in the upper part of their digestive tract. BURNETT (1966) demonstrated a good correlation between viscosity of barley extracts and chicken performance. Especially when barley was grown under arid conditions which results in a low enzyme content, growth in chicken was disturbed. Especially young birds were affected, displaying excess drinking and wet faecal droppings (see review by MUNCK 1968). BURNETT (1966) attributed the viscosity to the content of the highly viscous beta-glucanes in barley. These carbohydrates were hydrolyzed by beta-glucanases as demonstrated earlier by PREECE (1954). MUNCK (1968), however, working with barley meal suspensions in a Brookfield viscosimeter, pointed out that viscosity was dramatically increased with decreasing pH (Fig. 40, 41). In the peptic part of the digestive system of the bird, viscosity problems might arise because of the decrease in pH (see Chapter I). Large varietal differences in viscosity (Fig. 40) are found. The high-enzyme barley variety Kristina gives a much lower viscosity response than the variety Birgitta at different pH levels. The enzymatic effect on viscosity was proved by incubation of the meal suspension for 45 minutes at 25°C at pH 6.2 after which acid was added. The viscosity response at different pH's was thus completely suppressed with respect to Kristina and greatly reduced in Birgitta.

To test the effect of different enzymes on viscosity in barley, suspensions of pepsin and crude amylase were added (Fig. 41). At pH 2.0, pepsin had no effect on the increase of viscosity with time. At pH 3.3, however, pepsin greatly increased viscosity and reduced it again rapidly. The crude amylase preparation was still more efficient in depressing the increase in viscosity. Combination of the two enzyme preparations gave the most pronounced effects. The results

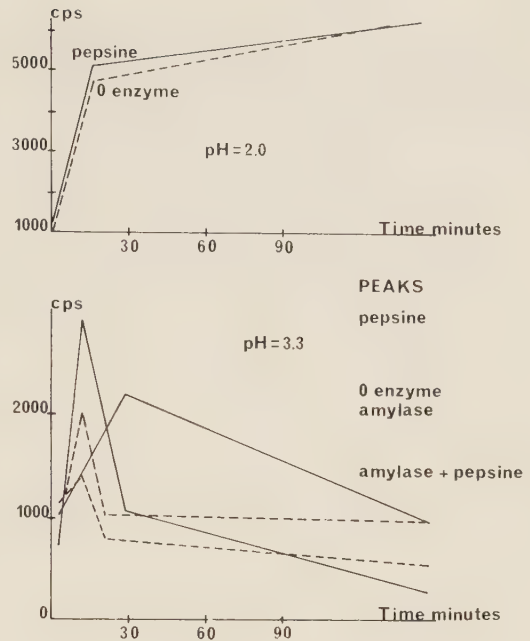


Fig. 41. Viscosity of barley meal suspensions at pH 2.0 and 3.3 with and without addition of pepsin and amylase (purum alpha + beta). Methodology as in Fig. 40 but different barley material.

were interpreted as follows: several proteins (e.g. prolamines) and carbohydrates (e.g. pentosans) are extracted at low pH's in high-molecular forms. This solubilization increases viscosity. Pretreatment with a wide range of enzymes from the barley itself, or from other sources, effectively suppresses the development of the high viscosity through a partial degradation of the largest molecules. It is unlikely that alpha amylase as such in the experiment in Fig. 41 contributed mainly to the reduction in viscosity. Other enzymes in this crude preparation may also be active. Thus, the beta-glucane-beta-glucanase system is but one of the factors involved in the viscosity complex. An increase in the alpha-amylase activity is likely to be correlated with an enhancement of other enzymes, such as proteases and peptidases, as well as beta-glucanase and other carbohydrases. Suspension-system studies *in vitro* should show a better correspondence with *in vivo* conditions than viscosity tests made on extracts.

BURNETT (1966) demonstrated the deleterious

effects of high feed viscosity for chicken by adding pectic acid, which greatly increases the viscosity of the diet. It is evident that birds are especially sensitive to a high viscosity per se. However, other effects from the increase of enzymatic activity in the barley seed than a decrease in feed viscosity might contribute to the improved nutritional value of enzyme-rich barley for birds. Enzymes especially from the enzyme-rich aleurone layer might digest non-available structures, which are prevalent in this region, release proteins and carbohydrates that otherwise would have been unavailable to the bird.

THOMKE (1972d) discusses several feeding experiments in Sweden and Denmark with barley at different ripening stages. In Denmark (BAELUM and PETERSEN 1970; PETERSEN 1969), chicken performance, in most experiments, slightly decreased from the yellow-ripeness stage to full ripeness. However, all experiments did not point in the same direction. THOMKE (1972c), obtained improvements up to 10 % of the productive value of barley to broilers at the late ripening stage over the early stage. He explained the main differences between the Danish and the Swedish experiments by the more extreme water contents and ripening stages of the latter. The use of different protein supplements — a composite with fish meal in Denmark, soybean meal in Sweden — may also have contributed to the differences observed.

EGGUM et al. (1969), in Denmark, demonstrated a decrease in lysine when barley was harvested overripe. The biological value and NPU for rats was accordingly lowered, while the results with regard to true digestibility were not fully consistent with time of maturity. Rat tests on the previously cited Swedish barley diets fed to chickens (THOMKE 1972c) resulted in significantly improved true digestibility, biological value, and NPU at late harvest. Wet storage (1–3 weeks) of the barley samples of different maturity results in improved performance for chickens and rats. For the latter, however, the latest harvest stored 3 weeks reduces true digestibility, biological value and NPU, indicating the existence of an optimum in treatment and confirming the results of EGGUM (1969). In the chicken trials (THOMKE 1972c), performance was optimal at the latest (combine) harvest and at 3 weeks of wet storage. Viscosity measurements on THOMKE's material, according to the previously cited method, result, however,

in a low-level plateau already at late yellow ripening and at one week of post-treatment, which may indicate that viscosity per se is not the major influencing character on chicken performance in this trial. The increased availability of protein and energy through the enzymatic effect is likely to be responsible for the improved nutritive quality in chicken, as was shown with regard to protein in the rat tests.

As was pointed out by MUNCK (1968), viscosity may only play a minor role for the nutritive quality of barley for birds when the barley is grown under the normally humid Scandinavian conditions, whereas this factor, according to BURNETT (1966), should be important for barley grown under extreme arid conditions, as in the Western United States.

Barley varieties with high (Birgitta) and low (Kristina) viscosity grown in Pullman, Washington (U.S.A.), under dry conditions and in humid Sweden and fed to mice display no major differences in gain, feed efficiency, water intake and faecal appearance (unpublished results), indicating that the increase in viscosity under the acid conditions in the stomach may be of minor significance in monogastric animals. However, BACHMAN (1969, 1969 pers. comm.) points out that pectins, which increase viscosity, have a positive effect in curing acute diarrhoea in swine. The pectins were added to a barley diet. Addition of acid in the form of betaine hydrochloride accentuates the curative effect. Already MALYOTH (1931) in his study on very young children convincingly proved the curative effect of pectins from apples in children's diarrhoea. Also other substances, like cellulose and pentosans (BACHMAN 1969), could exert a favourable effect to cure and prevent diarrhoea. Diarrhoea is widespread in swine production units in Sweden and Denmark and is of great economic significance. This problem has increased during the 1960's. The aetiology of the disease is difficult to determine, consisting as it does of hygienic (bacteriological), nutritional and genetic interactions (BACHMAN 1969). When the diarrhoeic syndrome develops, this is always preceded by a diminishing gastric activity leading to gastric stasis (WHITE et al. 1969). An increase in the viscosity in the stomach may stimulate gastric activity and counteract stasis. The cited investigations indicate that viscosity may be a limiting factor under certain stress conditions and that

the pH-dependent increase of viscosity in barley diets in the stomach, as previously discussed, may exert a favourable antidiarrhoeic effect. Late harvest and prolonged storage of barley at high water contents producing low viscosity at acid pH may thus contribute to the development of the diarrhoea syndrome in swine. Thus, the same barley-harvesting conditions that may be favourable for the nutritive quality of barley in chicken may be negative as regards swine owing to increasing susceptibility to diarrhoea in these animals. Further basic investigations in this field should be rewarding even for humans since diarrhoea is a major problem in mal- and undernourished children.

VI. Modification of nutritional quality through deterioration, processing and baking in cereals

1. Fungal growth and contamination

The interaction between cereal nutritional quality and the highly variable fungal flora constitutes a complex ecological problem with vast potential consequences with regard to economy in animal husbandry as well as with implications in human health. According to CHRISTENSEN and KAUFMANN (1969), the occurrence of mycotoxins in grain products has been a rather neglected field of research until the discovery of the aflatoxin complex in Great Britain at the beginning of the 1960's. Feed meal from peanuts contaminated with *Aspergillus flavus* resulted in widespread losses in various commercial animals. The causative agent was found to be a group of chemicals resembling coumarine, viz. of lactone or lactam nature. The aflatoxins, which are produced by *A. flavus*, are highly toxic with an acute effect in animals such as duck, turkey, monkey, swine, and rat, whereas mouse and sheep seem to be less sensitive. Aflatoxins cause liver necrosis and hepatic cancer in rats when fed chronically at as low a dosage as a few parts per billion in the feed. The toxin is not destroyed by cooking. It was found mostly in cereal crops mainly from the tropics, but also occurred oc-

casionaly in samples from the temperate zones. Toxin production is optimal during storage at 30°C at a relative humidity of 75–80 % (KRAYBILL 1969). In India and Africa ingestion of aflatoxin seems to be related to childhood cirrhoses in man (TULPULÉ 1969; KRAYBILL 1969).

KROGH et al. (1966) isolated *A. flavus* strains from Danish feedstuffs that were suspected of causing mycotoxicosis-like diseases in domestic animals. Danish *A. flavus* strains could produce small amounts of aflatoxins in, e.g., barley and rye compared with a known toxin-producing strain. It was concluded that aflatoxins only play a minor role with regard to the detrimental effects to husbandry animals in the samples collected in Denmark.

CHRISTENSEN and KAUFMANN (1969, p. 78) also conclude: "It seems highly probable that other mycotoxins are just as prevalent, and just as potentially dangerous, as aflatoxins." The authors review and exemplify mycotoxins that cause death in man and animals. KROGH and HASSELAGER (1968) and FRIIS et al. (1969) studied fungal nephrotoxicity in Danish swine. Mycotoxins caused degeneration of the kidney tubuli resulting in increased losses and consumption of water. Growth was severely hampered. The size and the colour of the kidney was also affected, enabling easy diagnoses in the slaughterhouses. Mold nephrosis is widespread among swine in Denmark, some years reaching up to 6–7 % of the finishing swine slaughtered. In dry harvest years, the disease seems to be up to 100 times less frequent than in wet years (PEDERSEN 1970, p. 25). FRIIS et al. (1969) obtained a very nephrotoxic substance, citrinin, from a barley sample, which could also be recovered from a *Penicillium viridicatum* strain isolated from the same sample. Studies of this sample (Sv 309) under the auspices of the Danish Grain Quality Committee (PEDERSEN 1970) revealed serious effects on swine, rats and mice (EGGUM et al. 1969).

It should be desirable to obtain valid estimates of the effects of the mycotoxins contained in feeds and foods on husbandry animals and human consumers in defined communities. The methodology for such an effort has still to be developed, and will without question require extensive interdisciplinary research (CHRISTENSEN and KAUFMANN (1969, p. 90–93)). Two main approaches may be followed. The first attempts to screen the status of the cereals produced from

the farms in inventories studying the fungal flora (WELLING 1970) and feeding characteristics (HUMMEL-GUMÆLIUS *et al.* 1969, 1971, 1972) with laboratory animals. As discussed in Chapter II, these inventories of barley and oats in Sweden point out that the seeds were stored on the farm at very high water contents even during dry harvest years. The cold winter period in Sweden, combined with storage in shallow layers or with proper ventilation of silos with air, prevented widespread deterioration during the autumn and winter months. Approximately 20 % of the storage samples, however, displayed a musty or acid odour. There was a tendency for reduced mouse gain on these samples. In 1969, outbreaks of mycotoxicosis in cattle in northern Sweden owing to moist harvest and storage conditions were recorded. Ten of these samples were fed to mice, three of which gave reduced gain and feed efficiency. It can be concluded from short-period experiments with mice that it is likely that only a minor part of the detrimental samples for husbandry animals will be picked up. The Swedish inventories are therefore now (1972) completed with veterinary investigations of farm animals fed cereals stored at high water contents.

The second major approach attempts to reproduce and study the deterioration effects in laboratory either by growing isolated fungal strains on autoclaved seeds and feeding them to laboratory and farm animals in search for the

active toxins (CHRISTENSEN and KAUFMANN 1969, p. 89–90) or by reproducing the ecological conditions for the complex growth and competition of different fungi in cereals samples that are then fed in turn to animals (HELLBERG and THOMKE 1969; THOMKE and HELLBERG 1970). The latter investigators obtained no toxic effects on feeding extensively molded barley and oats to swine, chickens (THOMKE 1972a) and calves. Mouse and rat studies on these samples indicate losses in available protein probably partly due to heat formation (THOMKE 1972b). These results indicate the difficulty in reproducing complex fungal effects and in generalizing from a limited number of experiments.

Another difficulty in studying the physiological effects of fungal toxins is their interactions with the dietary composition, e.g., the amino-acid balance, and with other environmental toxicants (KRAYBILL 1969). With respect to aflatoxin, this is understood from the action of the aflatoxin B₁ derivative on the DNA-dependent RNA synthesis thus inhibiting protein formation especially in the liver. Protein malnutrition is thus demonstrated to increase the sensitivity to low aflatoxin doses.

The importance of interactions of those widely different factors that influence the sensitivity of ruminants to deteriorated feedstuffs under Swedish conditions is further discussed by HOFUND (1967). Although conflicting information exists

Table 39. Acid preservation of barley: The effect on mouse* growth (g) fed barley treated with formic-, acetic- and propionic acid at different temperatures and water contents
Material from EKSTRÖM and THYSELIUS 1971.

Acid	H ₂ O (%)	High acid level			Medium acid level			Low acid level		
		0**	20°C	5°C	0**	20°C	5°C	0**	20°C	5°C
Formic	35	9.0	11.8	12.4	10.6	10.6	11.2	10.9 ¹	6.1 ¹	3.5****,1
	25	12.0	12.1	10.9	11.3	9.6	12.1	11.7	10.5 ¹	10.2 ¹
Acetic	35	10.6	9.1	10.7	10.0 ¹	6.8 ¹	11.2 ¹	11.2 ¹	6.4 ¹	9.0 ¹
	25	9.6	10.1	10.7	11.9 ¹	10.9 ¹	11.3 ¹	9.6 ¹	9.1	3.6 ¹
Propionic	35	10.8	2.5***	10.8	10.4	9.9	10.2	9.3	10.2	10.1
	25	11.9	8.9	11.2	10.9	9.6	10.7	10.2	9.3 ¹	10.5

¹ Mouldy as judged by visual inspection

* NMRI/Spf male mice, 11-day period (n = 4)

** Outdoor winter temperature

*** Damaged due to accident when drying

**** One animal. Five animals dead after 2–3 days

with regard to the importance of mycotoxins in practice, e.g., for husbandry animals in Sweden, and although it seems wise to carry out further specialized investigations with regard to the occurrence and effect of mycotoxins, the main efforts should be concentrated on preventing deterioration through proper harvest and storage technology. Besides conventional drying and aeration of bulks to prevent heat and water production by micro-organisms, other techniques such as refrigeration or acid treatment of high-moisture cereal stores are practised. EKSTRÖM (1972) compared propionic, acetic and formic acids in small amounts (about 1 %) added to barley seeds to prevent mold formation. With low dosages of propionic acid storage at high-water content could be carried through without fungal infection. Formic and acetic acids were less active. Barley (Table 39) was stored at 25 and 35 % water content at outdoor winter temperatures, 20° and 5°C. Three levels of acids were applied. The samples were fed to mice, and mold damage was estimated on the basis of visual inspection. It is seen in the table that one of the samples that was treated with propionic acid, 12 of the samples treated with acetic acid and 6 of the samples treated with formic acid were visually damaged by mold. Five of these moldy samples, treated with either acetic or formic acids resulted in strongly depressed mouse growth. It is seen in Table 39 that the negative effects of mold-damage seem to be quite randomly distributed among the treatments, each of which represents specific conditions for the development of different fungi and fungal products. The incidence of toxicity was surprisingly high in this laboratory experiment compared to that of the barley inventories.

2. Germination

Germination has been extensively studied by the malting industry (COOK 1962). According to the Swedish inventory previously discussed, germination proceeds in the spikes before harvest, especially in cool summers followed by a warm and rainy period in the autumn. Rye is particularly susceptible, but also barley and wheat can be heavily affected. The bread cereals, that germinate and show high alpha-amylase and low falling-number values, are classified as feed grade.

To study the effects of germination, rye, wheat and barley were placed in a moist chamber on wet filter paper and malted at 15°C. Rapid drying was carried out at 35°C. Complete amino-acid analyses (except tryptophane), with and without oxidation, were performed (Table 40); also mouse feeding trials were run (Table 41). The germinated samples were taken at the same morphological stage. The germination time (Table 40) was about 4 days (a shoot and 1–2 mm roots developed). It is seen from Table 40 that the essential amino acids lysine, threonine, valine, and isoleucine, are regularly increasing, as well as aspartic acid and alanine, whereas NH_3 , serine, glutamic acid, and proline are diminishing in all three cereals. On basis of the changes in amino-acid pattern as a result of germination, the potential nutritive quality of the cereal proteins should not be altered unfavourably. Rye, however, displays a smaller increase in lysine than wheat and barley; moreover, rye shows a slight decrease in cysteine and methionine in contrast to the other cereals. Wheat and barley display a prominent increase in aspartic acid compared with rye. The crude protein increases by 3–7 %, indicating a slight decrease in carbohydrates.

In the feeding trials with mice (Table 41) that were analysed with respect to carcass composition, also an earlier germination stage was included which comprised about 2 days of malting resulting in partially developed roots. It is seen that the nutritional performance of germinated rye is greatly reduced, even in the sample that germinated in 2 days. In wheat, however, this stage is not significantly different from the control, whereas growth and especially gain in fat is greatly reduced 4 days after germination. In barley, however, the 4 day sample was only slightly inferior to the control and the sample that was germinated in 2 days.

It can also be concluded from Table 41 that especially gains in fat and dry-matter consumption are reduced in the samples of rye and wheat involved. In barley there is no sign of a decrease in fat gain in any of the samples. PER and nitrogen retention are reduced in accordance with the decreased growth parameters in the different treatments.

This study verifies the results obtained by MUNCK (1965) using another strain of mouse (CBA). It was concluded that the slight decrease in available energy could not explain the sharp

Table 40. The effect of 4 days' germination on amino-acid composition (g/16 g N) of rye, wheat and barley

	Non-germinated g/16 g N (= 100)			Germinated (rel. values)		
	Rye	Wheat	Barley	Rye	Wheat	Barley
Lys	3.87	2.93	3.73	111	131	120
His	2.30	2.11	2.09	93	105	103
NH ₃	3.31	3.53	2.90	85	91	93
Arg	5.42	4.79	5.12	90	105	98
Asp	6.79	5.29	5.94	116	143	136
Tre	3.33	2.97	3.45	102	106	104
Ser	4.56	4.83	4.34	91	94	97
Glu	22.43	26.44	21.68	76	81	81
Pro	9.82	9.62	11.29	86	86	80
Gly	4.31	4.04	4.07	96	100	101
Ala	4.15	3.66	4.06	106	116	110
Cys*	2.35	2.37	2.28	90	98	105
Val	4.69	4.43	5.05	101	104	102
Met*	1.75	1.78	1.68	94	102	104
Ileu	3.57	3.47	3.57	103	104	104
Leu	6.26	6.64	7.13	97	99	97
Tyr	2.70	2.94	3.39	105	105	97
Phe	4.46	4.25	4.84	95	100	93
Sum (mmol/kg)	1002	889	911	98	99	103
Crude protein (%)	10.90	9.60	10.10	106	103	107

* Oxidation procedure

differences obtained. The changes in amino acid pattern presented in Table 40 could not explain the fundamental differences in reaction in feeding trials between rye and wheat on the one hand and barley on the other. MUNCK (1965) also studied the tendency of the fat to peroxidize, which could be expected to affect the feeding value. There was a low peroxide number in the milled malts. When meals were treated at 35°C overnight, peroxides increased greatly in the germinated samples. This was especially evident in rye, and could be due to the high content of linolenic acid in this cereal (Table 4). The controls were only slightly affected by this treatment. Vitamines, dissolved in water, were added to a small part of the diet (5–10 %) which was subsequently dried at 35°C. The vitaminized meal, minerals and other additions were then mixed in with the sample in dry form. Thus it is not likely that peroxidized fat would cause the great changes in nutritional quality discussed in Table 41 and by MUNCK (1965). A preliminary study with regard to the 5-alkyl resorcinols did not reveal any increase with respect to this potential factor. No mold formation was observed during

the germination process, and mold counts on agar plates were only moderately increased (MUNCK 1965). The slightly negative effect in nutritional quality in barley after 4 days of germination could be accounted for by a moderate decrease in available energy and proteins. The drastic reduction in nutritional quality in germinated rye and wheat could, however, not be construed on these grounds. Actually, it is believed that unknown dietary factors produced by the rye and wheat seeds during germination are responsible for these negative effects in mouse trials. Since germinated rye in the literature (see HOFLUND 1967; MUNCK 1965) often is considered toxic when fed to husbandry animals, a further exploration of the underlying principles is motivated.

3. Temperature effects

Deterioration through micro-organisms and insects may raise the storage temperature in mold-infected barley up to 70°C (HELLBERG and

Table 41. Feeding experiments with mouse on germinated rye, wheat and barley seeds

Diet	Dietary crude protein dry matter (%)	Gain per animal			Consumed per animal		PER	NR
		Total weight (g)	Crude protein (g)	Fat (g)	Dry matter (g)	Crude protein (g)		
Rye control	12.3	10.0 ± 1.3	1.74 ± 0.32	0.80 ± 0.10	50.1 ± 4.3	6.2 ± 0.5	1.63 ± 0.17	28.3 ± 4.1
Rye germinated (2 days)	12.8	4.4 ± 1.6	0.82 ± 0.26	0.19 ± 0.20	30.6 ± 5.7	3.9 ± 0.7	1.11 ± 0.25	20.6 ± 3.8
Rye germinated (4 days)	12.8	3.1 ± 1.0	0.61 ± 0.11	0.12 ± 0.12	33.9 ± 4.8	4.4 ± 0.6	0.69 ± 0.16	13.8 ± 1.2
Wheat control	10.6	8.3 ± 1.0	1.43 ± 0.25	0.95 ± 0.12	47.5 ± 5.0	5.1 ± 0.8	1.64 ± 0.08	28.1 ± 2.3
Wheat germinated (2 days)	10.0	8.6 ± 0.3	1.46 ± 0.11	0.98 ± 0.11	54.1 ± 6.9	5.4 ± 0.7	1.63 ± 0.21	27.8 ± 4.6
Wheat germinated (4 days)	11.2	4.0 ± 0.5	0.68 ± 0.09	0.03 ± 0.12	34.8 ± 3.0	3.9 ± 0.4	1.03 ± 0.03	17.9 ± 1.2
Barley control	11.3	10.0 ± 0.5	1.71 ± 0.11	0.88 ± 0.15	50.4 ± 3.1	5.7 ± 0.4	1.75 ± 0.08	30.0 ± 1.1
Barley germinated (2 days)	11.7	10.1 ± 0.4	1.71 ± 0.06	1.02 ± 0.11	52.3 ± 2.7	6.2 ± 0.3	1.64 ± 0.02	27.9 ± 1.0
Barley germinated (4 days)	11.9	8.5 ± 1.8	1.46 ± 0.47	0.91 ± 0.27	48.0 ± 8.3	5.8 ± 1.0	1.48 ± 0.11	25.0 ± 3.9

* Swiss × CBA male mice 2 × 4 per diet, 14-day experiment

THOMKE 1969). On the other hand, processes such as drying, cooking, and bread-making impose heat to increase storability, nutritive quality, and acceptability of the cereals and their products. During heating, reactive groups from free and bound amino acids and carbohydrates may react and change, e.g., the colour, taste, and nutritive performance of the cereal material. REYNOLDS (1963, 1965) reviews the chemical background of non-enzymic browning. The free epsilon-amino group of lysine is especially sensitive to heat in the presence of aldehyde groups, e.g., from reducing sugars. The first derivate formed is an aldosaamine, which is rearranged into a ketosaamine – the latter being unstable with reducing properties. By further heating, the ketosaamine is broken down to furfural derivatives and the amino group is deaminated. By conventional amino acid analysis, lysine that is bound is split during the acid hydrolysis and registered together with lysine with an unbound epsilon amino group. Because bound lysine seems to have an impaired nutritional availability, CARPENTER (1960) designed a method to measure the free epsilon-amino groups by a modification of the method by SANGER employing fluorodinitrobenzene. This method is, however, affected by carbohydrates and is unsuitable for cereal products and was further modified by ROACH et al. (1967) to fulfil that purpose. Extensive studies, mostly on proteins of animal origin, e.g., fish meal (MILLER et al. 1965), have been made to study the relation between heat treatments and changes in chemical composition and nutritive value.

In studies of heat-damaged, mold-deteriorated commercial barley samples, some of the samples were found to give a very low growth response in CBA mouse trials (MUNCK 1964c). However, with addition of 1–2 g lysine-HCL/kg, growth and nitrogen retention were almost identical with good quality barley controls. To study the nature of the heat damage in the laboratory, seeds were preconditioned at 4°C with various amounts of water and then transferred and tightly packed in plastic flasks, which were sealed and placed in a temperature-regulated oven (MUNCK 1966a). The treatments were 85°C for 24 hours (circles, Fig. 42, 43) and 67°C for 168 hours (squares). The untreated, unsupplemented barley sample is marked with a triangle.

Observe in Fig. 42 that gain in protein, gain in fat, protein consumption, and NR are reduced

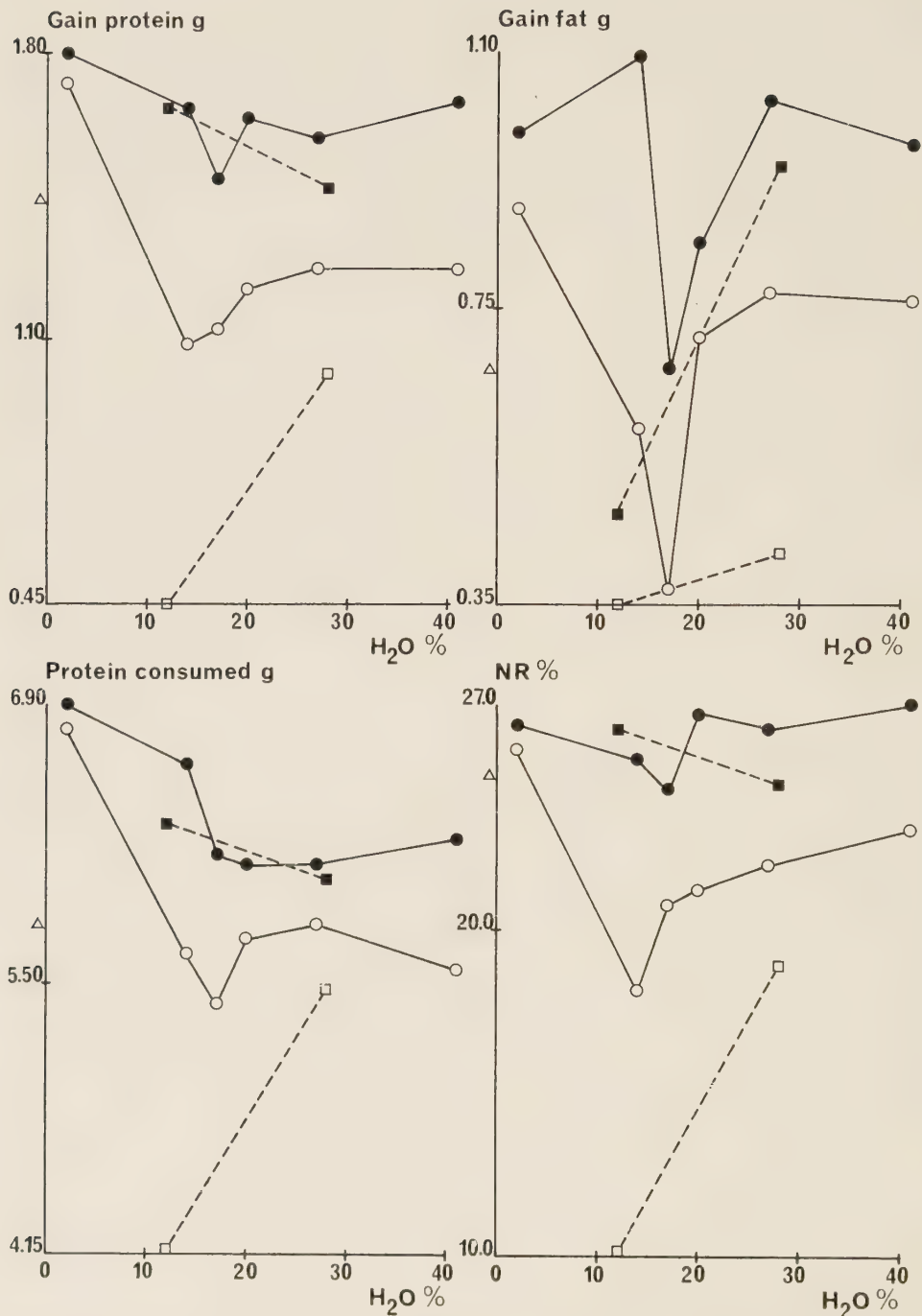


Fig. 42. The effect of heat treatments of barley seeds are recorded in mouse feeding experiments. Barley seeds are heat-treated at different water contents (2—40 %) in closed flasks at 85°C 24h (circles) and at 67°C 168 h (squares). The dried diets are fed without (open circles and squares) and with addition of 2 g lysine HCl per kg (filled circles and squares). Untreated control is marked as a triangle. Gain (g) in protein and fat, protein (g) consumed and nitrogen retention is related to different water contents at the heat treatment.

in the unsupplemented diets, especially around treatments at 15 % water content. This effect is most apparent in the long treatment at 67°C. Heating at low water contents (2 %) has a beneficial effect on the gain parameters. Adding lysine greatly increases gain in protein especially in the samples that were previously damaged in nutritive quality. After supplementation there is, however, still a pronounced minimum in gain in fat at treatments around 15 % water content, which indicates that there is a possible reduction in available energy with this treatment.

The data obtained indicates a preferential loss of the amino-acid lysine as a result of the heat treatment. This is verified in Fig. 43. Total lysine g/16 g N shows a pronounced minimum value at treatments of about 15 % water content. Thus, 67°C at 168 hours and 12 % water content results in depletion of lysine, viz. from 3.7 to 2.6 g/16 g N. These effects on lysine are studied by a conventional amino-acid analysis after HCl hydrolysis. The losses in lysine are due to deaminated epsilon-amino groups. In the reaction with an aldehyde group, e.g., from sugar, the epsilon-amino group of lysine may be nutritionally blocked by the formation of an aldosome derivative. This could be studied by the modified method for the estimation of free epsilon-amino groups of lysine in carbohydrate commodities (ROACH et al. 1967). The available free epsilon-amino lysine groups are represented in percentage of total lysine after treatment (Fig. 43). It is observed that there is a slight increase in unavailable lysine with treatments between 12 and 20 % water content. The differences are, however, very small (4 %), indicating that the conventional lysine analysis in this treatment imparts a fairly representative picture of nutritionally available lysine. It can thus be concluded that the reactions between the epsilon-amino groups of lysine and other reactive groups in cereals are, under the conditions discussed, completed and manifest an extreme change, which will show up as a loss of lysine in the conventional amino-acid analysis.

The treated samples were also studied with the dyebinding (DBC) method in relation to Kjeldahl protein. The DBP : KP ratios were markedly reduced for the various treatments and corresponded to the changes in lysine. At water contents of above 20 %, the treatments tended to give a relatively lower DBP : KP response than the

lysine analyses (Fig. 43). This can be attributed to enzymatic action which, degrades protein into fragments that are not precipitable by the dye, as has been demonstrated by MOSSBERG and MUNCK (cit. MUNCK 1968). The DBC method also gives an excellent correlation with the animal feeding test as expressed in gain in protein (Fig. 43). These results greatly contributed to the understanding of the DBC method as a screening test for available lysine, and not just as a substitute for Kjeldahl nitrogen determination. The methodology for the exploitation of the DBC method in plant breeding in the form of an estimate for lysine was developed further by MOSSBERG (1969, 1970) and is discussed in Chapter II in the present paper.

To study the effects of heat treatments on protein solubility in relation to nutritional performance in the 85°C series, a modified Osborne procedure was used (Fig. 44). The barley samples, milled in a falling-number hammer mill, were subjected to sequential extractions by water, 5 % K₂SO₄, 70 % ethanol, 0.1 % HAC, NaOH 1 (pH 9.0), and NaOH 2 (pH 11.0). The extracts were analysed for nitrogen according to Kjeldahl. Most fractions were greatly decreased at water contents of 11.4–39.2 %. The most basic NaOH extraction (2), at pH 11.0, was, however, increased, which reflects the denatured protein. No simple explanation reflecting the drop in available lysine in treatments around 15 % water content could, however, be offered from these results (Fig. 44).

Some of the most damaged samples were also subjected to pepsin digestion (Table 42). The degree of milling did not effect N solubility at pH 1.8 with and without pepsin in the control, whereas the heated samples required a more extensive milling for optimal yield of nitrogen, especially when extracted with pepsin HCl. Nitrogen solubility is generally affected negatively in the heat-treated samples, but there was no correlation with mouse gain or with lysine. The fractions from the digestive residue were treated with NaOH at pH 10. No residual protein was left after NaOH extraction in the unheated barley, whereas in the heated barley about 20 % remained in the insoluble residue. Also this factor failed to reflect the vast differences in nutritional values among the heat-treated barleys.

It is evident from the preceding experiments discussed previously that binding of the epsilon-

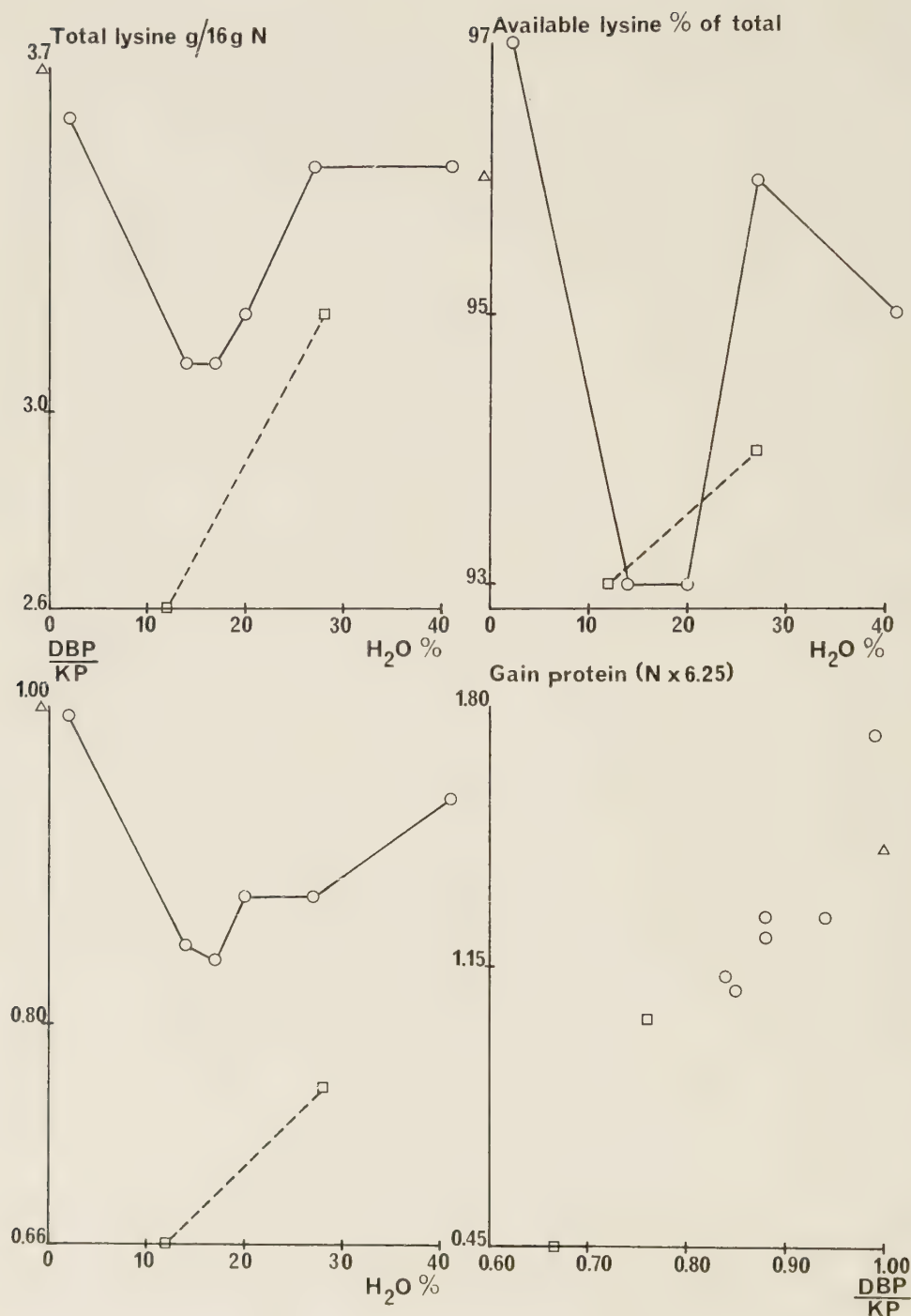


Fig. 43. The effect of heat treatments of barley seeds (Fig. 42) on total lysine g/16 g N, available lysine % of total, and dye-bound protein: Kjeldahl protein ratio (DBP : KP) as related to water content during heat treatment. Mouse gain in protein is also correlated with dye-binding (DBP : KP).

amino group of lysine in cereal protein depends heavily on parameters such as temperature, time of treatment, and water content. The structures of the different cereal proteins, as well as their compartmentization (see Chapter IV) with sugars, fats and acids, should play a major role for the degree of binding to the epsilon-amino group of lysine; however, this is difficult to assess and study. MILLER et al. (1965) studied mixtures of defatted cod fillet and glucose at different temperatures and water contents, under similar conditions and with comparable results to those previously discussed regarding the experiments with whole-barley seeds (Fig. 42, 43). In the cod fillet-glucose system, however, even low temperatures, e.g. 44°C at 9 hours affected lysine availability, whereas barley seeds maintained at this temperature for 2 months were unable to affect lysine availability as judged by mouse tests (MUNCK, unpubl.). This indicates that the activation temperature for lysine reaction and decomposition is higher in the structured barley system. Experiments with heat treatment (85°C for 24 hr at 28 % water content) of casein compared with the same casein dissolved at pH 7 and freeze-dried on wheat starch revealed heavy losses in total lysine in pure casein, whereas the same protein tied to the starch molecule withstands lysine losses (MUNCK and MOSSBERG, unpubl.). These results may be interpreted as starch preventing the casein molecules from forming bonds between free amino and carboxyl groups, the reaction of which is discussed by BJARNASON and CARPENTER (1969). Structure is obviously of

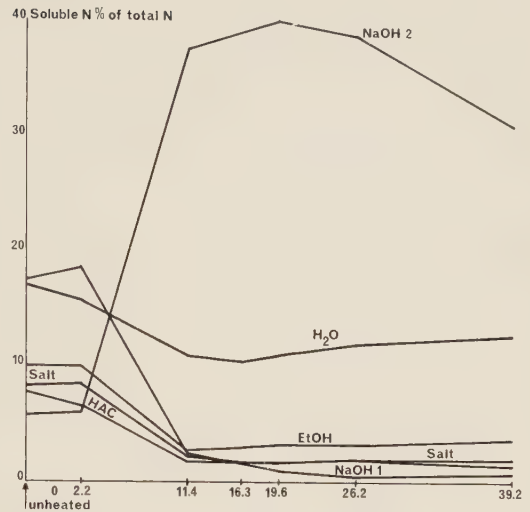


Fig. 44. Extraction fractions (soluble N % of total N) of meals from heat-treated seeds (85°C 24 h) from Fig. 42 and 43 as related to water content during heat treatment. For procedure see text.

major importance for the heat stability of proteins. The decreased fat gain and energy availability in the feeding experiment when barley was heated at water contents around 15 %, and where also lysine destruction was most evident, could tentatively be interpreted as due to carbohydrates trapped in denatured proteins that are difficult for the animal to digest.

Table 42. Pepsin digestion (0.0025%, pH 1.8, 16 hr) test of heated barley samples

Sample	% N-soluble in HCl at pH 1.8		% N Pepsin soluble 16 hr		Fractions from digested rest (milled once)		Mice gain	Dietary Lysine (rel. values)
	Milled once	Milled 4 times	Milled once	Milled 4 times	% N soluble at pH 10	% N insoluble after extr. pH 10		
Barley unheated	28.7	30.1	74.5	75.7	24.3	0.0	8.2	100
85°C, 24 hr at 26.2 % H ₂ O	16.5	15.7	46.5	51.0	25.3	23.7	7.5	85
85°C, 24 hr at 12.5 % H ₂ O	13.9	15.2	59.8	62.4	20.6	17.0	5.7	81
67°C, 168 hr at 10.8 % H ₂ O	19.3	20.6	60.3	66.5	16.2	17.3	2.5	66

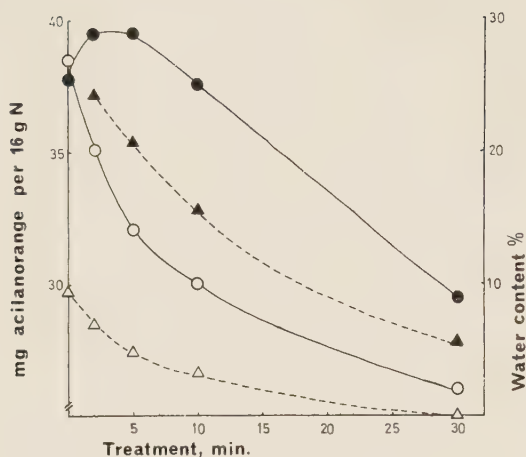


Fig. 45. Short-term effects of heat treatment (200°C; sand bath) on barley seeds studying dye-binding (mg Acilane-orange per 16 g N; filled circles and triangles) and water content (%) (open circles and triangles) as a function of treatment time (min.), and at two different initial (start) water contents 27 % (circles) and 10 % (triangles).

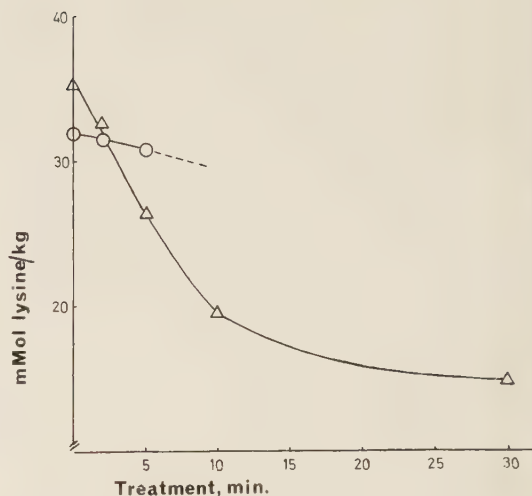


Fig. 46. The decrease of lysine (mMol/kg) in barley heated at 200°C in a sand bath (Fig. 45) at two different initial water contents 27 % (circles) and 10 % (triangles).

A. High temperature drying for cereals for feed

The fundamental data regarding barley previously discussed was further developed to study the possibility of effecting drying at high temperatures. The conventional cereal driers for seed for sowing and milling, utilizing warm air at temperatures up to 60°C, are constructed to ensure a viable seed with a good germinability. These driers occupy large volumes and their capacities are limited by a low diffusion velocity of the water vapor at these moderate temperatures. For cereals for feed, germination is not a quality factor of decisive importance. Thus, it should be possible to increase drying temperatures above the limit for viable seeds, but keep it beyond temperatures from which heavy losses of available lysine should result. It is also obvious that in an open system the temperature of the seeds is mainly independent of the temperature of the ambient heated air over 100°C as long as there is moisture left in the seed. The water in the seed thus acts as a thermostat and protects the seed from overheating even in very warm environments, provided the drying time can be made sufficiently short.

Preliminary studies (MUNCK and MOSSBERG

1967) in a small pilot plant demonstrated that barley could be dried in 10–15 minutes at air temperatures up to 175°C without loss of the nutritional value as indicated by mouse-growth tests (MUNCK 1968). The maximum seed temperatures were between 90 and 110°C, and the barley sample was rapidly cooled after drying. To study rapid changes in the laboratory, seeds were treated in a sand bath at 200°C (Fig. 45): the original water content here was 27% (circles) and 10 % (triangles). The water content (open circles and triangles) was rapidly reduced. The amount of dye bound per 16/g N is reduced by prolonged heating. Samples with an initial 27 % water content display, however, an increased dye-binding capacity at about 5 minutes of treatment, which accords with observations by MOSSBERG (1965, 1966). The initial denaturation is likely to expose new reactive groups that are able to bind additional dye. With regard to the decay of lysine (Fig. 46), the barley sample with low initial water content (10 %) rapidly loses lysine, whereas the other sample with 27 % water content displays a more stable lysine content. It takes 10 minutes to lower water content from 27 to 9 %. During this time, lysine and DBC are quite stable, indicating minimal impairment of the nutritional quality.

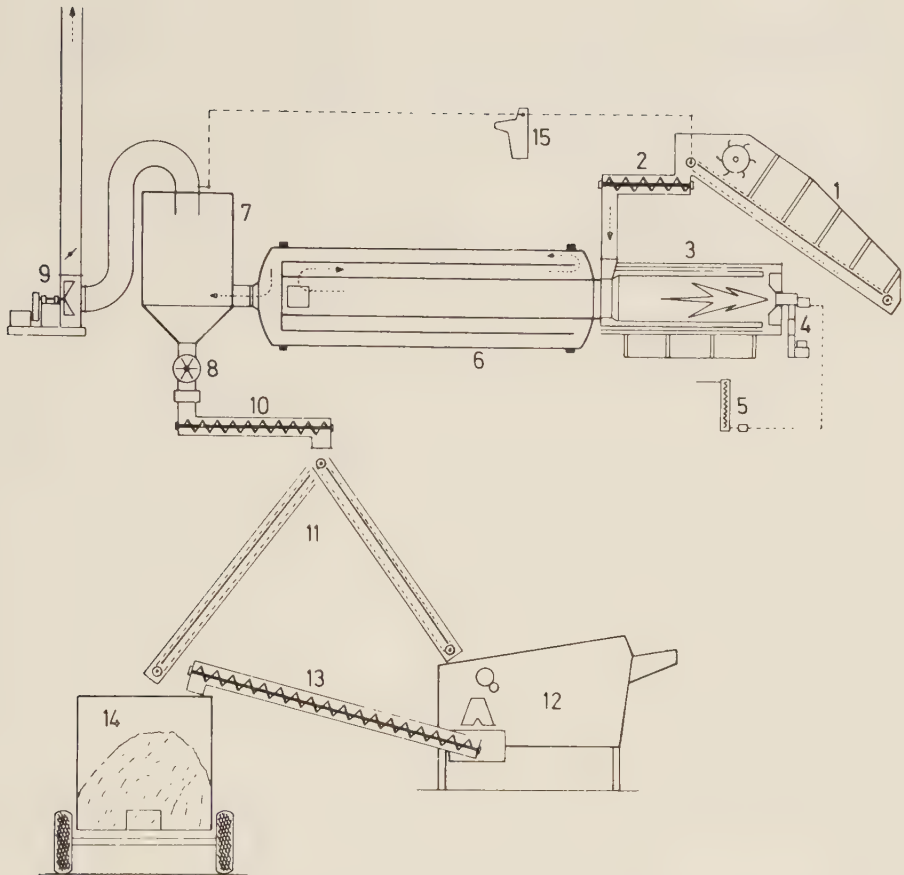


Fig. 47. Drying equipment Swiss Combi Mark II arranged either for drying of combined seeds transported (10, 11) after processing to a fan-cooled wagon (14) or for alternative drying of whole, chopped barley plants (straw plus seeds) the latter being transported (13) after threshing (12) to the wagon (14) for cooling. For a detailed explanation, see text.

High-temperature drying was first introduced in Sweden by EDELCRANTZ (1812). The drier consisted of a rotating drum that was driven by a steam engine: the seeds were moved through the drum with a screw. The drum was constructed of plates that were not completely tightened. It was placed in the smoke-gas channel leading from the steam engine. The wall temperature of the drum was 200–300°C, and that of the dried seed (bread wheat and peas) 90–100°C. The capacity was 1 ton of dried cereal per hour and the drying time was 13–38 minutes. For two years the machine was successfully tested in practice in Stockholm.

In Sweden, oil-heated drum driers for drying grass for pellets have been used for several years. Such equipment was studied with regard to its potential for drying seeds or unthreshed chopped plants (MUNCK and MOSSBERG 1971). The drier is equipped with a three-way drum driven by four synchronous electrical motors (Fig. 47). The maximum evaporation capacity is 2.5 tons of water per hour. The material for drying is transported (1, 2) into the drum (6), heavy oil is prewarmed (5) and pumped to the burner (4) attached to the oven (3). Material is passed through the drum (6) by the air stream produced by the flame (3) and the evacuating fan (9). A

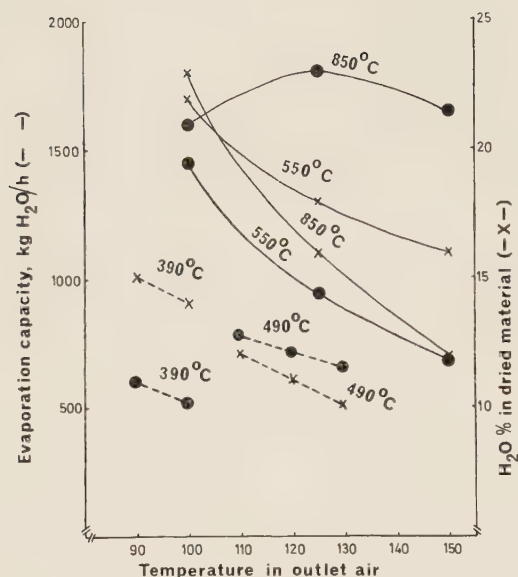


Fig. 48. High-temperature drying of barley with drier from Fig. 47. Barley seeds are dried combined (dotted lines) or as a part of whole-chopped barley plants (straight lines). Inlet air temperature is varied 390–850°C as indicated in the diagram. Evaporation capacity (kg H₂O per hour, dots) and water content of dried seeds (% , crosses) are related to temperature (°C) of the outlet air (Fig. 47: 9). Initial water content of chopped barley (seeds plus straw) is about 55 % and that of combined barley about 20 %.

cyclone separator (7) enables the materials to be transported away from the air stream through a sluice (8). In the case of the chopped, whole barley plant, the material is transported to a thresher (12). When combined seeds are dried, they are moved directly to a transport wagon (14), which could be cooled from the latticed floor by a cooling fan. The feeding of the drum with material is controlled with a temperature regulator in the cyclone (7) that is coupled to the switchboard (15) to give a signal to the material transporters (1, 2) when the temperature exceeds a given pre-set value on the control panel (15).

Under the very severe harvesting conditions, which occur in northern and central Sweden, before the snow sets in, barley must be harvested with water contents that are above 50 %. Under such conditions, the entire barley plant could be harvested with the chopper ordinarily used for grass. After drying, the chopped material could be threshed directly or it could be fed in toto to

ruminants. Under conditions when combining is possible, drying the whole seeds should be more economical if the straw is not utilized.

The possibilities discussed were tested (MUNCK MOSSBERG 1971) by drying whole barley plants at inlet temperatures of 850 and 550°C (Fig. 48, straight lines) or barley seeds at 490 and 390°C (broken lines). The chopped barley plant contained about 55 % water before drying; the seeds, about 35 %. The evaporational capacity at 840–890°C (inlet air) is independent of the temperature of the outlet air, whereas the capacity is greatly reduced at 530–560°C with an increase in the outlet air temperature. An increase in temperature in the outlet air, which is effected by setting the temperature regulator, greatly depresses the water content in the dried material. Oil consumption was 72 kg/hr at 530–560°C 120–150 kg/hr at 840–890°C. By using the chopper harvesting method, crushed seeds varied between 14 and 26 %.

The drying whole seeds (Fig. 48) was performed with barley with about 20 % water content. The water content of the dried material decreased from 15 to 10 % when the outlet temperature was increased from 90 to 130°C. The water evaporated was much less than with the chopped material, and only a fraction of the potential capacity of the drier was thus used. It was also shown that the transport capacity of the air through the drum was limiting for obtaining a high quantity of evaporated water. This was apparently due to the heavy grain material. By increasing the fan capacity and by giving the drum a more appropriate design, the drying capacity of whole seeds could be greatly improved. It was also shown by MUNCK and MOSSBERG (1971) that the temperature of the dried material was very well correlated with its water content. At a grain temperature between 100 and 110°C, a water content of 10 % was obtained. This correlation could be used for regulating processing. The material released from the drier thus contains ample amounts of calories that could be used for additional drying. At a water content of 16–17 % (at 70–80°C), the cereal material should be removed for cooling. This temperature allows 2–4 % water to be removed in the aerated wagon (Fig. 47: 14).

The dried material was analysed with the DBC method and fed to mice. No major effects could be observed, which was in agreement with the

pilot-plant experiment. If the drum was accidentally stopped, however, for a short period (10–20 minutes), a pronounced diminution in lysine availability was recorded. The economy for the machine for seed drying is very favourable when compared with conventional driers (MUNCK and MOSSBERG 1971). The equipment could be used in combination: drying grass and cereals over a 4–6 month period. The drying of sugar-beet tops and other offals could also be included. Capital costs per hour is thus greatly reduced when compared with other agricultural machines such as combines, which often are used only 50 hours a year. The high temperature drier could also be adopted to dry crop seeds without losses in viability but with reduced drying capacity. The equipment discussed here and by MUNCK and MOSSBERG (1971) is used regularly in two farm co-operatives in Värmland and Jämtland. The most northern drier (in Jämtland) processed in 1971 600 tons (outlet) combined barley with an average water content of 43 %. Similar results were obtained at the co-operative in Värmland. Both units are used for grass drying, which is greatly economized, since the net income from cereal drying solely defrays the total annual capital costs for the entire unit (total cost each about 700.000 Sw. Kronor or \$ 140,000). A similar technique used in Danish conditions is discussed by REXEN (1970).

B. Nutritional effects on protein due to baking

In Sweden, bread from rye and wheat in leavened form is mainly eaten in the form of thin cakes of hard-crisp bread, or as soft-loaf breads that often contain ample amounts of sirup and sugar. The first bread type is baked for a few minutes, while the latter types of bread often require up to 1 hour of baking time. With reference to the previous discussion regarding to the effect of heat on the availability of lysine in cereals, it is obvious that various factors in baking such as temperature, baking time, yeast and enzyme activity, sugar, additives, etc., may influence the nutritional quality of the protein in the bread. Supplements with protein and amino acids, as well as improvements in protein nutritive quality through plant breeding, may also be modified through baking.

Three different commercial breads were fed in mouse tests (Baking experiment 1, Table 43)

Table 43. Feeding experiment* with commercial breads. Baking experiment 1

Diet	Dietary crude protein dry matter (%)	Gain per animal		Consumed per animal		PER	NR
		Total weight (g)	Crude protein (g)	Fat (g)	Dry matter (g)		
1. Graham thin bread (soft)	16.4	11.9 ± 0.4	2.46 ± 0.26	1.27 ± 0.26	68.5 ± 3.4	1.06 ± 0.05	21.2 ± 2.8
2. Swedish wheat-rye loaf with sucrose	12.3	4.5 ± 0.2	0.86 ± 0.19	0.71 ± 0.12	55.4 ± 3.9	0.66 ± 0.03	12.5 ± 2.0
3. Norwegian loaf	13.3	7.0 ± 0.2	1.28 ± 0.05	1.03 ± 0.08	61.0 ± 9.2	0.88 ± 0.17	16.0 ± 2.3

* CBA mice 2 × 4 animals per diet, 20-day feeding period

Table 44. Baking experiment 2. Baking conditions

	% in dough ¹		Baking time (Min.)	Baking temp. (°C)	H ₂ O in cooled bread (%)
	Egg albumin	Water			
<i>High H₂O</i>					
1. soft	—	54.3	2.0	450—340—400	26.3
2. hard	—	54.3	3.6	430—340—380	11.0
<i>High H₂O</i>					
3. soft	2.3	53.2	2.0	450—340—410	29.4
4. hard	2.3	53.2	3.6	430—340—380	13.2
<i>Low H₂O</i>					
5. soft	2.3	49.9	2.0	450—340—410	24.6
6. hard	2.3	49.9	3.6	430—340—380	12.5

¹ Inclusively, 0.6% NaCl and 3.0% yeast

to study the variation in growth performance in the market. Vitamins and minerals were added to the air-dried bread meals. The graham thin bread displays the highest protein content and the best growth and nitrogen retention characteristics, presumably due to better amino-acid balance (whole meal) and short baking time. The Swedish and Norwegian loaves baked from meals with lower extraction grade were distinctly inferior to the graham thin bread in feeding characteristics.

The Swedish wheat-rye loaf, with high amounts of sucrose, usually up to 20% of the dry substance, was particularly poor as regards growth performance, PER and nitrogen retention (NR), indicating impaired lysine availability. LARSSON (1966), in rat trials, also noted the inferiority of the typical Swedish wheat-rye loaf as compared with soft-wheat bread. Both bread types gave response with lysine supplementation. Liver fat in rats was increased in comparison to the wheat bread when feeding the wheat-rye bread.

Further studies with wheat bread were made in a second baking experiment with respect to the effect of heat, water content and egg albumin supplementation on mice performance and amino-acid composition. In Table 44, the baking conditions are shown. Water was added at two levels during dough mixing. Dry egg albumin (2.3%) was added to one high-water and one low-water series, whereas one high-water series was run without supplementation. After fermentation, baking time and oven temperature were

varied at two levels to produce soft bread (2.0 min.) and hard bread (3.6 min.). The increased water content in the dough was reflected in higher moisture levels in the cooled bread. The addition of egg albumin results in increased water binding in the bread. The six different breads are shown in Fig. 49: the hard breads are darker than the soft ones. With regard to the browning reaction, the supplemented soft breads baked with high water content (3) are lighter in colour than the soft bread baked with less water (5). The opposite reaction is valid for the supplemented hard breads, with the high water level (4) displaying more browning than at the low level (6).

The amino-acid analysis (Table 45) generally shows decreased lysine values for the hard breads compared with the controls. Histidine, arginine, threonine, and methionine are less affected. The amino acid composition of the soft breads is changed very little through baking. A low level of water in the dough seems to give smaller losses in lysine and a lighter colour to the hard, supplemented bread (6).

The different bread types were fed to mice according to Table 46. Egg albumin was also added to the unsupplemented breads (1, 2) after baking. The soft breads were little affected by baking as compared with the controls. The hard breads gave, in general, lower gains and nitrogen retentions than corresponding soft breads, the differences being larger with respect to the unsupplemented breads. The breads supplemented

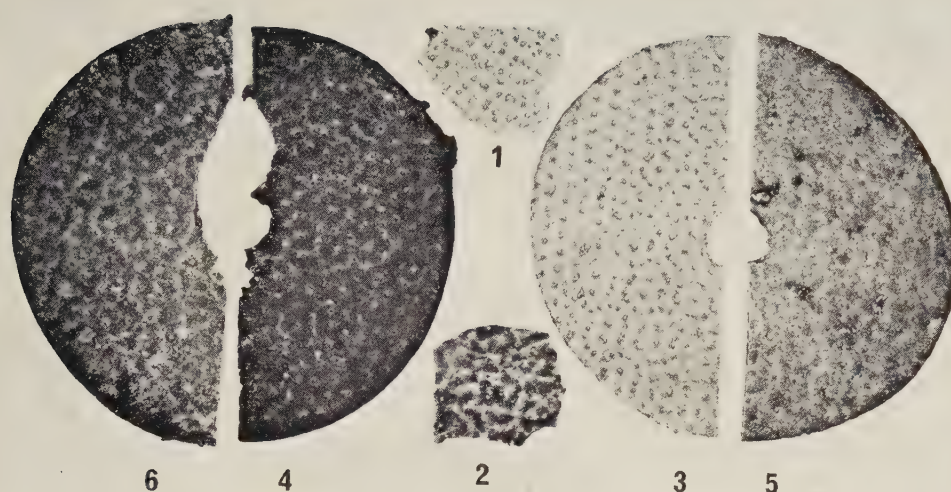


Fig. 49. Thin cakes from baking experiment 2 (Table 44).

Type of bread	egg albumin addition	water content in dough
1 Soft	—	High
2 Hard	—	High
3 Soft	+	High
4 Hard	+	High
5 Soft	+	Low
6 Hard	+	Low

Table 45. Baking experiment 2. Amino-acid analyses

	Amino-acid composition (g/16 g N)				
	Lys	His	Arg	Tre	Met
<i>No egg albumin during baking</i>					
Whole-wheat meal	2.82	2.42	4.77	3.15	1.63
1. Soft, high H ₂ O	2.95	2.49	4.70	3.27	1.50
2. Hard, high H ₂ O	2.30	2.19	4.23	3.10	1.41
<i>Egg albumin added</i>					
3. Soft, high H ₂ O	3.41	2.30	4.80	3.58	2.00
4. Hard, high H ₂ O	2.81	2.18	4.31	3.47	1.99
5. Soft, low H ₂ O	3.38	2.28	4.69	3.57	1.97
6. Hard, low H ₂ O	3.01	2.33	4.67	3.50	1.96
Wheat meal + egg albumin	3.40	2.32	5.11	3.67	2.17
Egg albumin	5.80	1.91	6.54	5.25	4.39

either before or after baking displayed a higher gain in fat than the controls which consisted of wheat-meal and egg albumin, indicating an improvement in energy availability with the baking process. There is no significant difference between the supplemental effect of egg albumin mixed in before and after baking, which indicates negligible losses with regard to the availability of the supplement. The difference in lysine between the supplemented hard breads baked at high (4) and low (6) water content in the dough is indicated in the feeding trials as a tentatively better performance on the latter diet (6). It is concluded that protein supplementation to hard crisp bread will still be highly available after baking. The water content in the dough will exert a minor influence with regard to protein availability.

In India chapatties—unleavened, thin soft-wheat breads—are baked with a short baking time. MATTHEWS *et al.* (1969) compared lysine supplementation to chapatties and to yeast wheat bread. Only 4 % of the added free lysine was lost while baking chapatties compared with 25 % in the conventional yeast wheat bread. From the nutritional point of view, an increase of free lysine or lysine-rich proteins in wheat, *e.g.* through plant breeding, will largely retain its availability in the baking procedures discussed.

Table 46. Baking experiment 2. Feeding experiment*

	Dietary crude protein dry matter (%)	Gain per animal		Consumed per animal			PER	NR	
		Total weight (g)	Crude protein (g)	Fat (g)	Dry matter (g)	Crude protein (g)			Lysine (mg)
No egg albumin during baking									
Whole-wheat meal	14.0	9.4 ± 1.2	1.75 ± 0.30	0.94 ± 0.25	60.0 ± 7.8	8.4 ± 1.1	234	1.12 ± 0.10	20.8 ± 1.7
1. Soft, high H ₂ O	14.1	9.6 ± 0.6	1.79 ± 0.14	1.05 ± 0.13	66.7 ± 4.0	9.4 ± 0.6	277	1.02 ± 0.08	19.0 ± 0.6
2. Hard, high H ₂ O	14.4	6.7 ± 0.5	1.16 ± 0.11	0.82 ± 0.19	57.2 ± 5.9	8.2 ± 0.8	188	0.83 ± 0.11	13.1 ± 1.5
Egg albumin added before baking									
Wheat meal + egg albumin	16.8	10.3 ± 1.0	2.02 ± 0.22	0.75 ± 0.03	60.1 ± 4.9	10.1 ± 0.8	335	1.01 ± 0.07	20.0 ± 0.6
3. Soft, high H ₂ O	17.2	11.5 ± 0.3	2.16 ± 0.05	1.16 ± 0.22	59.4 ± 3.2	10.3 ± 0.8	406	1.13 ± 0.08	21.2 ± 0.9
4. Hard, high H ₂ O	16.8	9.9 ± 0.3	1.78 ± 0.15	1.12 ± 0.25	62.0 ± 2.8	10.3 ± 0.5	292	0.96 ± 0.04	17.2 ± 1.4
5. Soft, low H ₂ O	17.5	11.1 ± 0.7	2.20 ± 0.10	1.02 ± 0.22	65.0 ± 2.5	11.4 ± 0.4	384	0.98 ± 0.09	19.4 ± 1.3
6. Hard, low H ₂ O	17.3	10.8 ± 0.6	2.04 ± 0.20	1.19 ± 0.14	62.6 ± 5.2	10.9 ± 0.9	307	0.99 ± 0.05	18.8 ± 0.9
Egg albumin added after baking									
1. Soft, high H ₂ O	16.3	10.6 ± 0.4	2.02 ± 0.06	1.12 ± 0.06	59.9 ± 5.3	9.8 ± 0.9	348	1.08 ± 0.09	20.8 ± 2.2
2. Hard, high H ₂ O	16.8	10.0 ± 0.3	1.89 ± 0.08	1.13 ± 0.06	63.9 ± 3.2	10.8 ± 0.6	301	0.92 ± 0.06	17.5 ± 0.7

* CBA mice 2 × 8 animals per diet, 20-day feeding period

VII. *Criteria for improvements in nutritional value — discussion and conclusions*

The varied concepts of *nutritional value* of feeds and foods are in essence operationally related to properties such as gain in meat in swine, and growth, health and longevity in human populations. Thus, the operational concept of barley has slowly evolved over a period of about 10,000 years as reflected in baking, cooking and brewing, as well as in animal feeding. In Sweden, for example, systematization in empirical agricultural research, comparing different feeds with barley, has resulted in a methodology to estimate the production results of feed composites employing one kg of barley as the ultimate standard (designated as a feed unit). This crude method to control input of feed resources in animals is still used as a convenient method among farmers. Basic animal and plant research, as discussed in Chapter I, has given us vast resources to analyse and study nutritionally functional factors such as amino acids, carbohydrates, fats, vitamins and minerals. These factors are offered to the consumers in the production chain in packages of various form and composition as represented, e.g., by barley, fish meal and soybean meal in feed production. It is now possible to interpret food and feed practices that are often already empirically evaluated. It is also possible to systematically change plant, feed and food composition through genetic and plant breeding methods, fertilizers, and processing.

Let us, as an example, consider future possibilities to breed cereals for improved content of the essential amino acids that are limiting for monogastric animals including man (Chapters III–V). About seven genes or mutants in barley and nine in maize are now known to favour synthesis of proteins that are high in essential amino acids and to depress lysine-poor prolamine proteins. Most of these genes seem to be associated with characters affecting seed morphology. This association, however, could be modified owing to alterations in the gene background, as judged by studies with the opaque-2 gene in maize and the lys gene from Hiproly barley (Chapter III). Further studies with respect to gene regulation of protein synthesis in developing endosperm will be of fundamental importance in setting the

extreme limits that can be reached by genetic and plant-breeding techniques. The ultrastructural recognition of how protein bodies and matrix protein are constituted (Chapter IV) will reflect changes in the concomitant mechanisms of protein regulation.

Nutritional studies of how various feed constituents and single proteins are degraded in the digestive system comparing, e.g., swine, chicken, sheep and laboratory animals will cast light on both the potential utilization value of cereals and the mechanisms of digestion and absorption (Chapter V). More information could also be gained as to how to interpret screening methods such as rat and mouse tests which are discussed in Chapter I (p. 12). Even if many of the genes studied will display negative pleiotropic side-effects in yield parameters and even if a few will be found to be incompatible with a high yield performance, data obtained from the opaque-2 and lys genes suggest (p. 52 and 44) that such negative effects might be completely eliminated by changing the gene background.

Recent data discussed in the present paper represent only the early stages of an accelerating development in the field of genetic protein engineering in cereals. High-yielding, high-lysine commercial varieties will certainly be released in the future. Their beneficial effects will be established in nutritional laboratories and in hospitals. The major question is, will the new varieties display an adequate nutritional role in feeds and foods in practice? It is imperative to understand how the new properties of high-lysine cereal varieties are to be recognized and utilized by various consumers and how the changing demands can be communicated to the grain farmers encouraging them to grow the new varieties.

There is thus no alternative to a thorough scrutiny through inventories of how cereals are selected, treated and used in society. The preliminary inventories (Chapter II) of barley and swine feed production in Sweden are aimed as a first step in developing a series of screening procedures that could be used versatily to study and appraise the effect of feed quality on animal production economy in a large scale. From such a material fruitful discussions with regard to priorities in research and development (e.g., regarding lysine in barley) comprehending the total production-consumption structure will be possible.

From the crude estimations discussed in Chapters II and VI, the usually humid harvest climate in Sweden was tentatively considered as one of the main factors affecting nutritional value of feed grains. Theoretically, a late-maturing, high-lysine barley displaying an increased risk of deterioration might be less economical in feeding than a normal-lysine, early-maturing variety that is more "resistant" to deterioration. The production is thus tied up with a complex of intermingled problems that for the most part cannot be solved independently.

Swedish agriculture, as an example, is characterized by:

- (a) Intensive cropping and mechanization
- (b) High labour costs
- (c) Small farms with high capital costs
- (d) Poorly developed co-operation between farmers
- (e) Uneven seasonal distribution of needs for labour, brief running time for machines owing to a short growing season, and unpredictable sowing and harvest conditions
- (f) Decreases in milk and beef production and increases in pork, eggs and poultry production
- (g) Unbalanced plant husbandry, both geographically and totally, with a deficit in high-quality (lysine) protein production and with a surplus in cereals
- (h) High food and feed price-levels owing to regulation and import customs to support farming incomes.

The multipurpose drying equipment for feeds presented in Chapter VI (p. 105) is *one* of the possible catalyzers for solving structural imbalance problems in Swedish agriculture. At present, there are 32 oil-heated grass driers producing a pelleted feed mainly for ruminants. This production represents only a small percentage of the entire forage harvest in the country. The drying stations are with few exceptions run as co-operatives, including the necessary field machines. Lucerne or grass is harvested 2 to 4 times a year at an early developmental stage. The pellets obtained contain more protein than normal hay and constitute an ideal fodder if supplemented with straw and cereals. Protein supplements, e.g., from imported oil cakes, are thus greatly reduced.

If cereals for feed and other crops (broad beans, fodder rape and sugar-beet tops or potatoes) are also considered for processing, a uniform utilization of management resources with a maximum insensitivity to unfavourable harvest weather could be obtained from May to November and from June to September in southern and northern Sweden, respectively. A great variety of feeds could be produced and the assortment is dynamically adjustable to the demands of the rapidly changing animal production without appreciably altering organization. Yield per hectare of specific crops in this system should not be considered separately. Uniform exploitation of processing resources and a high value of the *total* production from the drying station and related land should be the ultimate goals to obtain a favourable economic return.

If this cropping system were spread in Sweden, plant breeding should be directed towards considering yield and quality at different developmental stages and harvest times, supplying a wide variety of crops, namely annual and perennial grasses, winter and spring grains, legumes, and root crops. Both extremely early and late varieties of barley would have a place in such a system of production. The late varieties display an increased period of assimilation leading to increased yields, though less reliable if harvested by traditional methods. However, with the high-temperature grass drier, the whole barley plant can be harvested with the grass field chopper and dried as discussed in Chapter VI (p. 108). In favourable years, these late varieties could be combined and dried as seeds. Also crops that are marginally seed crops in Sweden, as the broad bean, could be treated similarly.

The integrated system is partly realized in two units in central (Molkom, Värmland) and northern (Östersund, Jämtland) Sweden. The two co-operative driers prolong the drying season with barley and oats harvested by combines. The cereals are regularly harvested at very high water contents (up to 50 %) even when ripe. In Molkom, broad bean and rape for fodder have been grown and processed in a small scale with good results. High-temperature drying of cereals for feed could favourably compete with conventional grain driers. The costs of drying whole barley plant (at 1200 hours of equipment use) is comparable with the costs of drying seeds in a conventional drier (500 hours of use) even if

straw is considered of no value and all the drying costs of straw are included in the costs of seed drying.

It is evident (MUNCK and MOSSBERG 1971) that the high-temperature, multipurpose drying technology will have an organizing effect, viz. promoting co-operation and efficient utilization of pooled resources of equipment, personnel and land – all with maximum reliability. Crop rotation to reduce the incidence of plant diseases will be greatly facilitated, especially with regard to the northern region. The increased assortment of high-quality feed products should favour local balance of plant and animal husbandry in a decentralized agriculture. On the other hand, feed factories could establish and manage large drying stations with local farmers as contractors. The industry might thus integrate feed production with better quality control and profit, but at the same time increase the present trend of centralization in Swedish agriculture.

In Denmark (Research Institute for Agricultural and Industrial Crops; FHI, Kolding: SONNE-FREDERIKSEN, HOLM-CHRISTENSEN, KIRK-CHRISTENSEN and REXEN 1967–72 pers. comm.) the use of high-temperature driers in agro-industrial systems has been further studied utilizing straw for alternative use in the manufacture of paper pulp, hard board and in an alkali-processed feed for ruminants.

Naturally, negative effects met with when introducing new techniques should be studied and eliminated before a new technology is adopted more widely. This is exemplified in the high temperature drying technique through the use of heavy oil which causes air pollution. This could, however, be eliminated by the use of low-sulphur oils or by gas. Fuel consumption could also be greatly reduced if the out-let warm air is recycled. In addition, alternative inexpensive fuels from waste products should also be considered.

Incomplete combustion of oil might lead to formation of the carcinogenic 3,4-benzpyrenes contaminating the feed (ROHRLICH and SUCKOW 1970; GORELOVA and CEREPANOVA 1970). The use of heavy metals as vanadium and lead in the petrol industry should call for specified certification of oils delivered to flame driers processing feed and food products. However, systematic research and control with regard to the benzpyrenes and heavy metals in this field in Sweden are not known to the author. In the future, there

is no room for omitting such considerations when new techniques are developed because resources are badly needed to “clean up” the already existing technology.

It is evident that an imbalance in protein production as it exists in Swedish agriculture could be corrected by many different means, as exemplified by the new drying technology or by breeding high-lysine barley, i.e., provided the innovations are accepted in the agro-commercial sector as overall profitable ones. High-lysine barley varieties could also tentatively economize feed mixing by bringing down processing costs and supplementation to a minimum. Local blending on the farm will thus be favoured reducing transportation costs.

In introducing new cereal varieties and management techniques, the crucial step is to *demonstrate* progress that is convincing to the participants in the production-consumption chain. The rapid introduction of nitrogen-responsive, widely adaptable wheat and rice varieties in Mexico and southern Asia (BORLAUG 1968) was possible even among illiterate farmers in certain areas with irrigation facilities. Farmers were convinced by radical yield response from 50 to 300 %. In countries with a well-developed agriculture like Sweden, 2 to 5 % stable increases in yield are sufficient for the release of a new variety. (Successful marketing in Sweden is carried out with reference being made to official trials and by advertisements and personal contacts.)

The problems associated with the introduction of high-yielding maize and wheat varieties and related techniques have been lucidly demonstrated by MYREN (1969) in his study of the Rockefeller Foundation programme in Mexico. Despite almost identical professional resources, increasing yield in wheat was much more successful than in maize. MYREN advanced two clusters of explanations based on social and plant characteristics and properties.

Social factors are associated with the fact that wheat is grown by a small number of commercially oriented farmers with irrigation resources, whereas maize is commonly grown by a large number of small, subsistent farmers that depend almost wholly on rainfall. Extension education is therefore a much greater problem in maize than in wheat agriculture.

However, probably the most important explanation for the greater success of wheat over

maize is due to differences inherent to the type of cereal itself. Wheat is a self-pollinating species, which can thus be maintained indefinitely by the local farmer. On the other hand the hybrid maize that had been introduced had to be purchased each season, necessitating an efficient seed-multiplication agency as well. The new wheat varieties were also easily bred for resistance to a defined disease complex, whereas the disease pattern in maize was much more difficult to overcome by breeding. Furthermore, the new hybrid maize varieties displayed poorer ecological adaptability than the new wheat types and therefore a much more varied assortment of new varieties were needed in maize to cover the country than in wheat.

A new maize project (CIMMYT 1969), which changed to open-pollinated varieties and concentrated on adaptability, was introduced founded on the above-cited experience. These maize varieties could be multiplied by the farmers themselves.

Quality parameters that exert their influence later in the production chain are much more difficult to demonstrate and purvey on the market, as compared with the yield character. The consumer must be able to recognize the improvement, the consequences of which must be fostered through the various steps of production and distribution to the farmer as quality payments that are determined by a simple and inexpensive screening technique.

This principle can be illustrated by the wheat and rye quality programme for southern Sweden (Skåne, Fig. 50). Because of humid harvest conditions, bread-seed quality was very adversely affected in the 1950's and early 1960's. Cereal was imported to satisfy the demands of consumers for a large volume wheat-bread loaf and high quality rye-crisp bread. At Svalöf, a breeding programme (HAGBERG and PERSSON 1969) was introduced for low alpha-amylase content in rye. The falling-number method was further studied and adopted as a screening technique (OLERED 1967), and is used today for payment to farmers for quality grades of wheat and rye. Quality-grade wheat (1971) resulted in a premium payment of 2 %, whereas low falling-number analyses gave a maximum price reduction of 21 %. To stimulate production of high-quality bread seed, an information service (Fig. 50) has been organized that relies on data

from both weather forecasts and current local analyses of falling number. Thus, the farmer is able to adjust his combine and drying resources for harvesting at the optimal time for obtaining maximum seed quality and premium payments for his seed. The quality control programme, combined with the introduction of sprouting-resistant rye varieties, enabled a self-sufficient supply of Swedish-grown rye for the crisp-bread industry.

The control system discussed, which is suitable in industrial countries, is naturally of limited applicability in developing countries, where there is a radically different level of organization. Consumer-quality characters in cereals, such as baking quality in wheat and maize and cooking quality in rice, however, still have a major influence on the price on the market in the developing countries. Thus, red wheat in India accounts for an approximately 20 % lower price than yellow-coated seeds solely on basis of less favourable consumer acceptance (SWAMINATHAN et al. 1969). Thus, certain apparent characteristics of foods and their raw materials are retained on the market as a result of the selection dictated by conservative specifications of the consumers. The means of production of major food commodities are, however, much more easily altered than the consumer products themselves.

When introducing high-lysine barley varieties in Sweden, the economic advantage of the cereal must be demonstrated in official trials and in practice in individual production units. If uncontrolled factors, such as mycotoxins (Chapter VI), exert a major influence on animal performance, the beneficial effects of a change in amino-acid composition will be more difficult to assess in practice. Under such circumstances, a quality payment programme might even be unfeasible because of the various effects of interaction. We could thus arrive at an "overcontrol" situation where administration and investments exerted for control of a quality parameter do not correspond to the gains involved. Therefore, sound data from specially designed inventories of the different production steps are needed to effect the appropriate judgements and to bridge over possible conflicts among members of the production-consumption chain.

When the beneficial effects of the new, high-lysine barley variety have been established in practice, the problem remains as to how this



Fig. 50. Map presenting the organization of the quality control service for falling-number of wheat and rye in Skåne (Scania), southern Sweden. The farmers co-operative (SLC), which mainly purchases the seeds, is organized into a head office (square), regional offices (triangles), commercial offices (large filled circles), elevators (small open circles) and local units (small filled circles). Samples from wheat and rye from about 100 farms are analysed regularly at The Swedish Seed Association, Svalöv and the information from the trend in the falling-number quality is co-ordinated with the weather prognosis in a forecast available in the regions through automatic telephone answers, the telephone numbers of which are included on the map. Thus, farmers can consult daily these telephone answers to obtain advice with regard to optimal harvest time for quality and delivery to the retailers (From extension service paper *SLC Falltals/Väderprognos 1970/71*, *Skånska Lantmännens Centralförening U.P.A. Malmö, Sweden*).

variety is to be distinguished and separated from other barley varieties and how the increase in the economically limiting factor is to be analysed for feed optimization. In the inventories, an over-supplement of lysine-rich protein in industrial feed composites owing to a lack of analytical guidance was tentatively found (p. 33). Individual analyses of raw materials would probably spare extra supplementation of expensive fish protein for insurance of nutritional quality. The

introduction of high-lysine barleys without matching, appropriate screening techniques probably would result in an increased variability of lysine in complete feeds and would thus in turn even result in a wastage of high quality protein.

The Kjeldahl method of analysing crude protein would be unsuitable as a guide for the proper use of high-lysine barley in a mixed market containing both normal and high-lysine varieties. The dye-binding method (DBC), being even less

expensive than the Kjeldahl analysis, could serve as an ideal screening method for stimulating improved quality through a premium-price programme, as previously discussed with respect to baking quality. The DBC method could also record negative effects on protein quality owing to deterioration and processing (Chapter VI p. 103).

In large parts of the United States where maize is grown the soybean is an economically important cash crop that is utilized in the oil industry, as well as being an important protein source for feeds. In cattle the use of urea has, to a large extent, been a substitute for soybean meal. However, new protein products derived from soybean, and used in the paper and food industries, have captured new markets. It is not likely that high-lysine maize will be able to function as a substitute for the multipurpose oil-protein role of soybean. However, increasing amounts of maize are being processed for the industrial use of starch in the United States with protein as a by-product. If high-lysine maize varieties displaying a wide range of different starch characteristics are bred, development of high-lysine protein products from maize may have profitable "spin-off" effects.

Specialty devised inventories with regard to cereal quality as related to harvest, storage and consumption practices seem to be indispensable also in developing countries for establishing priorities in integrated programmes, including medical and health investigations. Deterioration effects resulting from insects and fungi during harvest and storage in rainy periods are indicated (p. 97) to cause unfavourable interactions that could be nutritionally limiting. In the Philippines, rice is harvested at a moisture content of 20–35 % and has to be rapidly dried for safe storage in the warm and humid climate. High-temperature, short-time drying technique (IRRI 1969), similar to those described in this paper (p. 107), have been investigated to improve drying.

In developing countries, the cultivation of high-lysine cereals for food is a much more complicated and diverse problem than introducing high-lysine barley in Swedish feed rations. The previously cited difficulties in introducing high-yielding (normal-lysine) maize hybrids in Mexican agriculture (MYREN 1969) would be still more accentuated if opaque-2 varieties were to be promoted among subsistence farmers. Con-

sumer objection to the opaque seed colour would hamper utilization in foods. However, recent breeding progress (VILLEGAS 1972) has resulted in almost completely modified, hard, vitreous seeds of the high-lysine mutant still carrying the expression for protein quality. Governmental agencies could control high-lysine maize grown on large farms delivered and marketed through state and private distributors in cities and towns. Convenient checks could be effected with the dye-binding analysis, for which a simple, battery-operated field instrument could be developed.

On the other hand, if the high-lysine cereal is not characterized by some easily recognizable morphological trait or characteristic, difficulties will arise in its distribution and use especially among the subsistence farmers, control and extension service not being available in the scale needed. The new, high-yielding, high-lysine maize varieties are likely here to be mixed and intercrossed with normal lines, and thus will not impart a stable contribution to human nutrition. Morphological markers regulated by the government could tentatively be introduced on the plant or seed level of *all* high-lysine varieties released. These characters should be chosen not to interfere with acceptability. Still extension work to the subsistent farmer is an immense undertaking with an introduction of a "package" of locally adjusted simple techniques, conferring benefits that he could personally recognize and exploit.

It is evident that several characters of the cereal plant beyond nutritional quality, as determined in laboratories and hospitals, are essential for its overall nutritional value in the society structure. The essential new information that must supplement our present knowledge is linked with how man recognizes, selects, utilizes and controls items such as cereals for food and feed, and how the collective action of his preferences and rejections affect his social and physical environment.

It is obvious that the communication and retrieval of information in man, obtained through his different senses, a specialized brain, and through language, constitutes a feed-back system that imposes restrictions and deliberations upon his future actions. *Apparent values*, such as colour, texture, smell, taste, foodmaking properties, and social image, guide food selection and

determine plants that are to be collected and grown. The apparent values are slowly related by an accumulation of knowledge as to their implications (evaluation). Examples of *implicated values* are a feeling of satisfaction after a meal, growth of children and longevity in population.

It is beyond the scope of this paper to go deeper into the science of the *value theory* as presented by RESCHER (1969). However, the value theory may offer a fruitful methodology for value systematization and elucidation.

The natural sciences seem, according to RESCHER (1969, p. 49–53), to have suffered heavily from ideas from the philosophers SPINOZA and LOTZE: “that the value point of view is somehow at odds with that of our scientific understanding of the world.” While LOTZE differentiated between the realms of *fact* and *value*, later scientists (see RESCHER 1969) claim that value experiences have an objective basis in being either appropriate (correct) or inappropriate (incorrect). RESCHER (p. 10) further points out that; no sharp line of separation can be drawn between statements of value and statement of fact: values theses are always shot through with factual considerations founded upon a vision of how life ought to be lived.

The concept of *cereal value*, e.g., in the cereal production processes could be looked upon as a collection of working hypotheses that are used by individuals, institutions, and industry in different contexts to fulfil widely different purposes, sometimes resulting in conflicting and uncompromising interactions. Values could be manifested in different ways. Thus as pointed out by RESCHER (1969, p. 2–4), a person subscribes to certain values through his active discourse and thinking, to be in turn expressed through his behaviour in overt action. The degree of conformity in these dispositions can be studied in two types of inventories comparing analyses through “the tools of inquiring” and the “budget analysis, i.e., a scrutiny of patterns of resource investments (material, time, energy, effort, toleration for inconvenience, etc.)” respectively.

In introducing high-lysine maize varieties to subsistent farmers in the developing countries, we aspire to change their implicated value concept of maize to correspond to the nutritional effect of maize plus milk or maize plus poultry. Because man is not able to smell or taste essential amino acids in cereal protein form, indirectly

visible, apparent value characters (or analyses in sophisticated societies) must be attached to the varieties. When the farmer is able to recognize the variety from others, he might later associate it with improved nutrition and health of his family or to increased performance in animal feeding. To be able to catalyze such a breakthrough, the plant breeder and his associates must find a methodology to evaluate *how* the production chain selects and transforms the cereal raw material and what this signifies for the consumers. The directors for such a programme have to create their own vision of how life should be led and how plants and society should be optimized to accommodate their vision. They must then be equally successful in introducing this ideal in the plant as in the social fabric of the community. As a starting-point inventories are necessary that combine the tools of the social and natural sciences, e.g., studying the degree of conformity of apparent and implicated values in a farming community relating these to actual living conditions. Such an investigation, is in fact a study in survival and selection in the Darwinian sense.

In *The Variation of Animals and Plants Under Domestication*, DARWIN (1868) lucidly describes (Vol. I, p. 10) how he arrived at the concept of natural selection through his studies of variation among species selected by man. We might similarly focus on how man acts as a *selector* utilizing his *value apparatus* as a selection instrument, exploiting the variability of plants and animals, tools, energy, and labour to favour his living and survival. The value apparatus of man represents:

- (1) A rationalization of his basic physiology
- (2) A rationalization of his action to underwrite evaluation comparing apparent values of items prior to selection with their implicated values after consumption.
- (3) A unique laboratory for logistic experimentation nurtured by a continuous search for extremes and similarities, splitting and recombining concepts from the most diverse sources as a basis for experimental behaviour.
- (4) An efficient methodology to establish new behavioural patterns embodying a sustained rationale of planning and purpose adding to the arsenal of means for satisfying living and for future survival.

While man is engaged in appraising, selecting and evaluating, he continuously "molds", e.g., his cultivated cereals, swine, and other economic plants and animals, his diet and behaviour—his environment. The vehicle in this overall process is his values apparatus used as a selection instrument among the items in focus for *his* benefit. It is evident that the results of intelligent selection is greatly dependent on:

- A. The efficiency of the screening and control methods, including the human senses.
- B. The availability and variability of items, properties, values, etc. that serve as a major source of inspiration for human innovation.
- C. The widespread implications of the apparent values that are used as limited selection criteria but which might have vast consequences evaluated in ecological and social terms.

The previously cited work by DARWIN (1868, Vol. II, p. 192–249) elucidates just as much of the *values* of man as it describes to us *facts* with regard to the development of plants and animals for agriculture and of fancy birds and flowers. DARWIN personalized the selection force in the form of selection by man, realizing its dynamic power reflected in the recent husbandry of plants and animals. In the spirit of DARWIN, we should thus consider a high-lysine barley plant not only as a sample of *Hordeum vulgare* but as product of human creativity cast in the form of an improved cereal. Selection was to DARWIN (1868 Vol. II p. 248 line 36–p. 249) a much more evident part of evolution than the physiology of organisms and their inherent variation, however, the necessity of the latter for evolution was repeatedly acknowledged by him (Vol. II p. 250–270). It appears that the major avenue of research in the natural sciences since DARWIN has been mainly investigations of fundamental chemical and physiological properties and their variation in living organisms with emphasis on description of partite elements and systems. These properties stand in a much more clear perspective to the present generation of natural scientists than the effects of selection—either natural or artificial. The selection force of some four billion human beings acting individually and in groups on the various resources and the ecology of our planet has, however, only more recently been more fully and generally acknowledged and appreciated

by scientists. Selection and exploitation by man of world resources is now regarded as the most singular and important component of natural selection and survival with respect to all living organisms.

Scientists must recognize the operational character of the human value apparatus—the most potent selection force in nature. DARWIN (1868, Vol. II, p. 239) wrote: "It is an important principle that in the process of selection man almost invariably wishes to go to an extreme point."—Evidently, this methodology of polarization in selection is necessary to obtain information in all operational systems relying on pure empiricism. However, it is indeed an expensive method in terms of resources and lives. To obtain dynamic equilibrium in ecology for the survival of mankind, new solutions (coping with overpopulation, food and energy shortages, social distress and war) relying on a *synthesis in research* must be coded and transferred to suit the value apparatus of people using them as legitimate selection criteria favouring survival and quality in life.

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